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Founded by William J. Gies in 1919

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Editor

HAMILTON B. G. ROBINSON

APRIL, 1948

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International Association for Dental Research
Twenty-Sixth General Meeting
June 19 and 20, 1948
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THE EDITOR'S VIEWPOINT

Bruckner (p. 128) manufactured dental plaques for dialysis studies by immersing cellophane strips in saliva. He then proceeded to study the passage of bicarbonate through the membranes. The plaques did not prevent the passage of bicarbonate but did slightly inhibit such passage. Sodium bicarbonate was then demonstrated to inhibit *L. acidophilus* in vitro. In a few individuals (eighteen for two months and nine for six months) sodium bicarbonate dentifrice tended to lower acidophilus counts. Further controlled study is essential.

Howell, Schlack, Taylor, and Berzinskas (p. 136), report further on their careful study of the effect of oxalates on dental caries in experimental animals. Previously, it had been found that oxalates offered protection against decalcification of rats' teeth by acids produced in beverages. Potassium oxalate administered in drinking water of white rats on corn meal diet had no apparent effect on the incidence and extent of carious lesions. For the present report, the effects on dental caries of feeding various amounts of potassium oxalate in the drinking water of cotton rats was observed. Again, no effect on the incidence or extent of carious lesions was observed. The hope that oxalates might offer a simple method for control of dental caries has not been borne out by the experiments of the group at the Naval Medical Research Institute as reported to date. It is imperative that carefully controlled experiments of this type be used before materials are recommended for general use by the public, lest false hope be given to members of the profession and to the public.

Muhler and Van Huysen (p. 142) report further on their studies of fluoride reagents and powdered enamel solubility. We are still of the opinion that powdered enamel may not give the same results as may be obtained by the use of substances on surfaces of teeth, and that the laboratory work should be accompanied by clinical studies. It is interesting to note, however, that their experiments show that a 2 per cent solution of sodium fluoride is the best concentration to use in reducing solubility of powdered enamel. Two per cent solutions, likewise, have been found effective in reducing caries in groups of children. Muhler and Van Huysen showed that buffered solutions of sodium fluoride are more effective than unbuffered solutions in reducing powdered enamel solubility, and that the pH of 4.5 is most effective for a 2 per cent reagent solution in reducing the solubility of powdered enamel.

Davies, Slack, and Tilden (p. 149), have utilized a media devised by Wade, Smiley, and Boruff for estimating the numbers of acidogenic bacteria. The method has been tested on a small group and showed fair correlation with clinical data, but there is still need for further study with large groups of individuals. The principle of the method is to use a media which supports the growth of many different types of bacteria but permits differentiation of strongly acidogenic organisms from weakly acidogenic organisms and nonacidogens. The media uses bromcresol purple as an indicator. Further developments along this line should be useful to investigators of the carious problem and may have wide clinical application for rapid differentiation of caries-susceptible and caries-resistant individuals.

Braunschneider, Hunt, and Hoppert (p. 154), using the Michigan State College strain of genetically resistant and susceptible rats, have found that resistance to caries is partially dependent upon age. The teeth in the animals were significantly more resistant to decay at 100 and 150 days than they were at thirty-five days. These animals which have been standardized by controlled breeding should be of value in a number of experiments of this type in which certain variables can be eliminated by the use of a group of animals of known caries susceptibility. Of course, this type of study brings up another point, namely, that many earlier studies on dental caries in rats must be reviewed in view of the work of the Michigan State College group which has shown that caries in rats may be related to their genetic background.

Gilda and Goldberg (p. 161) have devised methods for determining the size of interproximal cavities. The authors have improved on Shepherd's method for cavity area and propose a method for volume measurement by weighing. Previously, Dahlberg (*J. D. Res.* **19**: 479, 1940), had suggested volume studies in a general way. The methods suggested have value for studies of the progress of caries. However, most caries control programs and are aimed at preventing the initiation of caries, not at controlling cavity size, and properly so.

A paper by Eggers Lura appeared in Volume 26 of the JOURNAL OF DENTAL RESEARCH and discussed phosphates in saliva. Davies and Rae, of Toronto, take issue with some of Lura's findings and suggest the use of another method for determination of salivary phosphates. They believe that the results with their method are more uniform than those of Eggers Lura and that their organic phosphate fraction is much smaller than that presented by him. In order to present a valid discussion, the note from Davies and Rae was submitted to Dr. Eggers Lura, of Denmark, for reply. His reply is published along with the original note. In his reply, Lura points out that his technic gives greater value for inorganic phosphate and, especially, for total phosphate, and that both procedures are open to considerable experimental error. He does not attempt to decide which method is the better of the two. Such discussions are sometimes valuable contributions. When these are valid and adequately presented, the Editor will consider such communications for publication in the JOURNAL OF DENTAL RESEARCH.

Klein and Kramer (p. 170) describe a form for recording and machine tabulation of statistical analyses. Anyone who has attempted to carry out clinical investigations on a scientific basis realizes the value of a simplified, rapid, reproducible method that does not distract the examiner from his task. The authors have adapted the mark sense principle, using electrographic pencils and a special machine.

James (p. 175) presents a subject which has not previously received consideration in dental research. A careful study was made of 1,319 consecutive cases in which local anesthetics had been given and the data were correlated with the weather conditions in the city of Beverly Hills. The results are rather interesting and suggest that minor toxic manifestations occur in association with drops in temperature, and abnormal anesthetic reactions are associated with temperature changes. James proposes that the temperature drop produces toxic reactions and the temperature rise produces incomplete anesthesia. The author points out, in his discussion, that these results may be true only for his particular geographic area. It might be well for individuals in other parts of the country to keep such records and make comparisons. This study illustrates the fact that one in dental practice and without the assistance of a large clinic can carry out clinical research on a statistical basis for profitable scientific evaluation.

Montelius (p. 187) studied characteristics of the skull and teeth of Arikara and Hopi Indian skulls. He found great similarity between the teeth of the Arikara and Hopi group although there were some fairly marked variations in the characteristics of the skulls. Montelius suggests that, as far as these two groups are concerned, the dental patterns are conservative traits. He further proposes that the dental patterns may be used as criteria for determination of racial groups. One interesting observation of the author is that the American Indian may have developed prominent cheekbones because he used his anterior teeth to a great extent, developing the masseter and internal pterygoid muscle rather than the muscle groups more prominently developed in other racial groups.

Kitchin and Robinson (p. 195) give the results of abrasive tests on nine widely used commercial dentifrices and ten experimental dentifrices. The abrasiveness of dentifrices was tested on the root surface of extracted teeth and the depth cut per 100,000 strokes determined. The abrasiveness of the dentifrices was quite marked when measured on root surfaces. A table is presented which shows the period of time necessary to cut halfway through the cervical area of an upper canine tooth with available commercial dentifrices. This "half-life" table suggests the need for careful evaluation of dentifrices before

they are recommended to any patient with exposure of the cervical areas of the teeth.

Glickman (p. 201), continuing a study reported in the February issue of the JOURNAL OF DENTAL RESEARCH, presents the effect of acute vitamin C deficiency on the response of periodontal tissue to artificially induced inflammation. Inflammation was induced by application of 10 per cent silver nitrate in the gingival sulci of vitamin C-deficient and control guinea pigs. Although pocket formation and necrosis resulted in both the control and the vitamin C-deficient animals, the destructive changes in the underlying tissues were more severe in the vitamin C-deficient group. The results of this experiment suggest that, while vitamin C adequacy will not prevent inflammation from local causes, it may aid in limiting the destructiveness of locally induced periodontal disease.

Buchbinder and Schwartz (p. 211) used absorbent points impregnated with 3,000 units of crystalline sodium penicillin in the treatment of forty-four cases of infected root canals. Twenty-three cases were sterilized with one treatment, six with two treatments, and three with three treatments. Nine cases required four to six treatments, and in three instances, penicillin-resistant organisms were present and the root canals were not sterilized at all. The authors believe that this method offers an improvement in root canal therapy. They believe, too, that the use of penicillin-impregnated points is preferable to other methods of placing penicillin in the canal. They admit that instances are encountered in which penicillin-resistant gram-negative organisms are present. Buchbinder and Schwartz propose gram stains of cultures as sufficient for recognition of such organisms.

The proceedings of the Michigan Conference on dental caries control are continued with papers by Hine on prophylaxis, tooth brushing, and home care, Fosdick on the degradation of sugars in the mouth and menadione, Kesel on the effectiveness of dentifrices, mouthwashes, and ammonia-urea compounds, Hill on the use of penicillin, and Arnold on the effectiveness of ingested fluorides. These studies together with those published in the February issue and those in the forthcoming June issue will present a rather complete story of the present status of dental caries control. The authors have carefully selected the chaff from the wheat and present the real food in their papers. While many of these reports appear to have a negative tone, it should be remembered that there has been too much optimism surrounding the use of some methods of dental caries control. The note of caution, which was found throughout this conference, is one which can well be injected into many discussions of dental caries before dental societies and in dental journals. At the Michigan Conference the discussions were recorded by a stenographer and are published as edited by Dr. Kenneth Easlick, Dr. Philip Jay, and the Editor. Only the principal papers were submitted to the authors, as it was considered too laborious to submit all discussion to the many individuals concerned and it was desired to present the material as given at Ann Arbor rather than with any marked revisions. An effort has been made to retain the spirit of the conference. This has resulted in inclusion of an occasional facetious remark and the use of short conversational phrases rather than literary contributions.

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IN VITRO STUDIES OF SODIUM BICARBONATE IN RELATION TO CERTAIN FACTORS CONCERNED WITH DENTAL CARIES

ROBERT J. BRUCKNER, D.D.S.

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FOR many years sodium bicarbonate has been employed as a dentifrice on a more or less empirical basis. An investigation has been undertaken to acquire information concerning the nature of the behavior of sodium bicarbonate in relation to various factors concerned with dental caries. The following report deals primarily with in vitro studies that have been carried out.

DIALYSIS OF SODIUM BICARBONATE THROUGH BACTERIAL PLAQUES

Bacterial plaques have been of great interest to investigators of dental caries. The plaque, it is generally agreed, is a thin, adherent film, white and cloudy in appearance, present on improperly cleansed tooth surfaces, and consisting of microorganisms and debris that is primarily of organic nature. It cannot be removed from the tooth surface by a stream of water or by gentle swabbing,[1] but can be removed from accessible surfaces by vigorous brushing, especially with the aid of a mild abrasive.[2]

Attempts to study dialysis through plaques have been made, various methods being used to obtain the plaque or plaque material. The plaque may be removed from extracted teeth by immersion of the latter in dilute acids, the plaque thus allowed to float free. The structure obtained may be used as a dialyzing membrane, although the difficulty in handling such a delicate film without producing minute, if not gross tears, is apparent. Dobbs[3] studied dialysis through plaques obtained in this fashion, and Pincus[4] made a similar study of the permeability of Nasmyth's membrane. In addition to the use of dilute acids to remove these structures, they were treated with other reagents. Dobbs employed alum to prevent the proteins from swelling, whereas Pincus removed the mucin entirely with the aid of N/10 NaOH. Because of the changes that these treatments bring about, it was felt that such liberties should not be taken with the chemical and bacterial organization of the plaque if a justifiable in vitro study is to be made.

Plaques have also been "made" by suspending, in water centrifuged particles of material scraped from the surfaces of teeth.[5] Such a method does not preclude the possibility that bits of calculus, materia alba, or other material, are collected with the actual plaque material. More important is the fact that the plaque is not a conglomeration of large particles that adhere to the teeth, but rather, a thin film whose thickness is best measured microscopically.

The manufacture of plaques outside the oral cavity has also been accomplished with the aid of an elaborate apparatus designed to simulate the ebb and

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flow of saliva, in a study of artificially created carious lesions.[6] Plaques have been formed in vitro on extracted teeth, glass slides, and inanimate smooth surfaced objects simply by immersing of the object in saliva that was changed at daily intervals.[2]

After consideration of the various methods of study, the plan adopted was to immerse sheets of cellophane in saliva and to deposit thereon a bacterial plaque. Since cellophane is a dialyzing membrane, it is a simple matter to ascertain the rate of dialysis of a solution, such as sodium bicarbonate, through it, and compare the amount of dialysis that takes place with the amount that occurs through a similar sheet of cellophane covered with plaque. Any major difference between the rates of dialysis could be ascertained and attributed to the presence of the plaque. Experimentation with the plaque would thus be simplified, drastic changes due to chemical treatment and mechanical manipulation of the plaque would be avoided, and, in general, a close duplication of naturally occurring plaques would be expected.

METHODS

1. Production of Plaques.—Sheets of cellophane about 5 cm. square were immersed in saliva which was changed daily, and incubated at 37 degrees Centigrade. A squat, wide-mouthed jar served as a container. Every day the sheets of cellophane were transferred to a fresh bottle which had been filled with sufficient saliva (paraffin stimulated) to cover the several sheets immersed. Before incubation was begun, a pinch of sucrose was added to the saliva to insure nourishment for and growth of acidogenic organisms. Within a few days a thin film, whitish in color, was readily seen to have been developed on the cellophane. In the present study, the plaque was allowed to develop, by the method described, for a period of three weeks. The film that resulted adhered to the cellophane to the extent that fairly vigorous rinsing with distilled water did not remove it, although superficial debris washed away readily. The plaque could be rubbed off with the fingers, however. Scrapings from the plaque-covered cellophane that had been rinsed with water were treated with Gram stain, and various types of organisms common to the oral cavity were observed. Bacilli, cocci, and thread-like organisms were seen, as well as yeasts, the latter probably more numerous than might be expected.

2. Dialysis.—The dialysis experiments were carried out as follows: A glass tube was cut into three lengths of about ten inches each. These were sealed at one end. The other end of each tube was fire polished to remove sharp edges and produce a rounded lip. The diameter of the mouth of the tubes measured from the crest of the rounded lip was 6.9 millimeters. An equal volume of sodium bicarbonate solution was placed in the respective tubes and the open end of two of the tubes, each covered with a sheet of cellophane. The sheet was folded up against the sides of the tube and firmly held in place with a tightly wrapped rubber band. The mouth of the third tube was covered with a sheet of cellophane on which plaque had been deposited. The three tubes were then suspended over a beaker of running water with just the tip of the cellophane-covered end immersed. Fig. 1 represents this arrangement with only one of the three tubes shown.

While dialysis was taking place, the following procedure was carried out. A volume of the bicarbonate solution, equal to that which had been placed into each tube, was titrated against approximately N/10 HCl. It is not necessary to know the exact concentration of the acid as long as the same solution of acid is used for the titrations before and after dialysis. Methyl red was used as the indicator. The amount of acid necessary to neutralize the original bicarbonate solution was thus established. Dialysis of the bicarbonate solution in the tubes was stopped at a specific time and the solution of each tube was titrated with the N/10 acid. On the basis of the difference between the volumes of acid used before and after dialysis, the percentage of dialysis that occurred could be calculated readily.

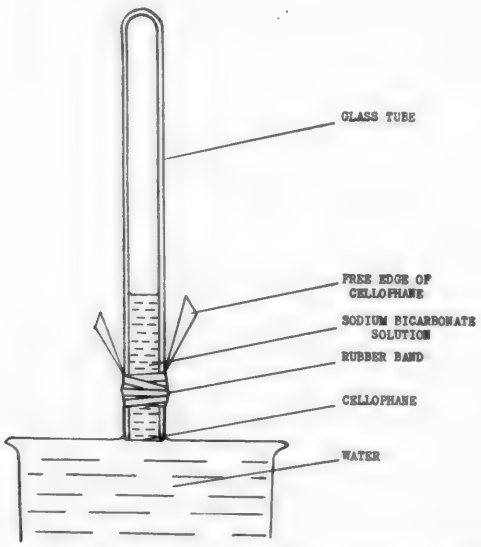


Fig. 1.—Arrangement of apparatus for studying rate of dialysis of a sodium bicarbonate solution through a membrane. Diagrammatic.

Titration of the original bicarbonate solution was carried out at least two times. In titrating after dialysis, the bicarbonate solution was poured from its tube into a beaker, a procedure which leaves some bicarbonate adherent to the tube walls. When the end point was nearly reached, therefore, the solution was poured back into the tube to wash it clean of bicarbonate, and the titration was then continued in the beaker once again. It was determined that a small amount of the bicarbonate is lost through adherence to the tube and cellophane. This loss amounts to between 0.5 and 0.9 per cent.

DISCUSSION

The first row of figures in Table I indicates that 2.0 c.c. of a 0.5 molar solution of sodium bicarbonate was neutralized by 9.70 c.c. of acid before dialysis. After 2.0 c.c. had been dialyzed for 180 minutes through plaque-covered cello-

phane (indicated as "plaque" in the table), 6.70 c.c. of acid were necessary to bring about neutralization, which indicates that 30.9 per cent less acid was required for the latter titration or connotes that 30.9 per cent of the bicarbonate dialyzed through the membrane. In comparison, the solution that was allowed to dialyze through cellophane alone for the same length of time (and which is listed as "control" in Table I) required only 5.70 c.c. of acid to complete the titration. To ascertain whether the difference between 6.7 and 5.7 is simply within experimental error or whether the difference is definitely due to the presence of the plaque, is the purpose of the second control. It will be noticed that, in this case, the amount of dialysis that took place through the cellophane membrane covering the control tubes was 41.2 and 43.3 per cent, respectively, a difference of 2.1 per cent, whereas the difference between the controls and the plaque-covered cellophane is 10.3 per cent and 12.4 per cent, respectively. In all cases, the least amount of dialysis took place through the plaque-covered cellophane.

TABLE I

COMPARISON OF AMOUNT OF SODIUM BICARBONATE THAT DIALYZES THROUGH CELLOPHANE MEMBRANE AND THROUGH PLAQUE-COVERED CELLOPHANE MEMBRANE; DIAMETER OF ALL MEMBRANES, 6.9 MM.

SOLUTION	DURATION OF DIALYSIS (MINUTES)	VOLUME OF SOLUTION (C.C.)	NUMBER OF C.C. ACID			% DIALYZED
			BEFORE DIALYSIS	AFTER DIALYSIS		
.5 M	180	2.0	9.7	plaque	6.70	30.9
				control	5.70	41.2
				control	5.50	43.3
+.5 M	20	2.0	11.1	plaque	9.90	10.8
				control	9.50	14.4
				control	9.20	17.1
.5 M	28	2.0	9.7	plaque	8.95	7.7
				control	8.60	11.3
				control	8.65	10.8
.5 M	32	2.0	9.7	plaque	8.95	7.7
				control	8.50	12.4
Saturated solution	30	2.0	25.7	plaque	22.75	11.5
				control	21.90	14.8
				control	20.60	19.8
Saturated solution	30	1.0	12.3	plaque	10.50	14.6
				control	9.80	20.3
				control	9.65	21.5

It will be observed that the time of dialysis is relatively short, varying from twenty to thirty-two minutes, with the exception of the 180-minute experiment. The reason for carrying out the dialysis for this short span of time is associated with determinations made in the oral cavity, with the aid of an antimony electrode, of the length of time that bicarbonate is retained in the mouth and exerts its maximum alkaline effect.

It may be concluded from these experiments that sodium bicarbonate will dialyze through bacterial plaques but that there is some inhibition of the rate of dialysis. The evidence here presented does not establish the absolute rate of dialysis through the plaque, for the actual rate is governed by the cellophane on which the plaque is deposited. It is believed significant, however, that the amount of dialysis is less through a plaque-covered sheet of cellophane than

through a similar sheet of cellophane, of equal dialyzing area, that is free of plaque material.

It is felt that this method of studying plaques offers an opportunity to delve further into the sugar chemistry of these bacterial films, as well as to study certain physical phenomena. An investigation is under way which, it is hoped, will shed more light on the role of the plaque and its bacterial inhabitants in the process of dental caries.

EFFECTS OF SODIUM BICARBONATE ON ORAL ACIDOPHILUS

To determine the effect sodium bicarbonate might have, above and beyond that of alkalinity, on the production of lactic and other acids by *L. acidophilus* in a carbohydrate broth medium, the following experiment was carried out.

METHODS

A dextrose broth was employed ("Difco" Bacto-Dextrose Broth, Special) which, after preparation and autoclaving, has a pH of 4.9. The broth was divided into 5.0 c.c. portions and these were adjusted to various pH levels as indicated in Table II, using sodium bicarbonate on the one hand and sodium

TABLE II

AMOUNT OF ACID PRODUCED BY L. ACIDOPHILUS IN CULTURE BROTH ADJUSTED TO VARIOUS pH LEVELS WITH SODIUM BICARBONATE AND SODIUM HYDROXIDE

INITIAL pH	24 HOURS				48 HOURS			
	SOD. BICARB.		SOD. HYDROX.		SOD. BICARB.		SOD. HYDROX.	
	TYPE 1	TYPE 2	TYPE 1	TYPE 2	TYPE 1	TYPE 2	TYPE 1	TYPE 2
8.0	8.4	9.1	7.4	7.5	8.9	9.4	7.6	7.5
c.c. NaOH	–	–	0.25	0.2	–	–	0.15	0.2
7.7	8.9	8.9	7.3	7.5	9.1	8.8	7.4	7.3
c.c. NaOH	–	–	0.1	0.1	–	–	0.1	0.1
7.4	8.4	8.0	7.0	7.1	5.4	8.0	5.6	6.9
c.c. NaOH	–	–	0.05	0.1	0.4	–	0.3	0.1
7.1	8.2	8.1	6.5	6.7	8.8	9.0	4.7	4.1
c.c. NaOH	–	–	0.15	0.17	–	–	1.0	n.r.*
7.0	8.3	7.7	6.4	6.7	8.5	8.3	4.7	6.7
c.c. NaOH	–	–	0.1	0.15	–	–	1.0	0.05
6.2	4.3	5.8	4.6	5.9	4.0	6.0	4.8	5.9
c.c. NaOH	0.6	0.05	0.4	0.75	0.95	0.05	0.9	0.05
5.6	4.5	5.4	4.9	5.5	3.6	5.3	4.0	5.3
c.c. NaOH	0.6	0.05	0.4	–	2.0	0.05	1.2	0.05
4.9*	4.1	5.0	4.1	5.0	4.1	4.9	4.1	4.9
c.c. NaOH	0.35	–	0.35	–	0.45	–	0.45	–

The pH of the broth is 4.9 when made up as directed. Neither sodium bicarbonate nor sodium hydroxide was added to the portions tested at this pH level.

*Indicates no reading.

hydroxide on the other. *L. acidophilus* of types 1 and 2 were used. The adjustment of the pH of the broth with sodium bicarbonate was complicated by the fact that autoclaving the broth thus adjusted apparently produced some sodium carbonate and liberated carbon dioxide to the extent that the pH of the

broth was raised to about 9. The broth was, therefore, sterilized first and adjusted afterward by the addition of a measured amount of sodium bicarbonate solution to each 5 c.c. portion of medium, the quantity to be added having been determined previously by trial adjustments. In addition to the possibility of contamination, such a procedure introduces a pipetting error, which, however, is believed to be not greater than ± 0.1 of a pH unit.

The various 5 c.c. portions of broth were then inoculated with either the type 1 or 2 *L. acidophilus* and the tubes containing this inoculated medium were divided into two groups which were then incubated at 37 degrees C. for twenty-four and forty-eight hours, respectively. At the end of the indicated time the pH of the cultures was determined and the extent of acidogenesis established. The latter was carried out by titrating with N/10 sodium hydroxide, bringing the pH of each culture, in which acid formation had taken place, back to its original level. The relative quantity of acid formation that took place is shown in Table II below the corresponding pH reading, on the lines labelled "c.c. NaOH." At the end of twenty-four hours 5 c.c. of a bicarbonate-adjusted broth with an initial pH of 6.2 and inoculated with type 1 *L. acidophilus*, gave a pH reading of 4.3 and required 0.6 c.c. of N/10 NaOH to return the pH to 6.2.

The organisms were inoculated from stock cultures with a platinum loop wire so that the number of organisms introduced to each 5 c.c. portion of broth was approximately the same.

DISCUSSION

The results of this experiment, tabulated in Table II, can be summarized briefly if the media be divided into two groups: (1) the tubes of broth adjusted to an initial pH below 7.0, and (2) the broth adjusted to an initial pH at or above 7.0. In the lower pH ranges the sodium bicarbonate showed a slightly smaller inhibitory effect on acidogenesis than did the sodium hydroxide. In the higher ranges the bicarbonate apparently evolved carbon dioxide and underwent other changes so that a strongly alkaline reaction resulted which counteracted any acid formation that may have occurred. It must be observed, however, that in those portions of broth which had been adjusted to alkaline levels with sodium hydroxide the amount of acid production was very slight. This is to be expected since the optimum pH for these organisms is in the range of about 5.0 to 6.5. It may be concluded from this that the inhibition of acid production by sodium bicarbonate, at pH levels that are optimum for *L. acidophilus*, is no greater than that of an alkaline agent such as sodium hydroxide.

To ascertain the effects of sodium bicarbonate on the growth of *L. acidophilus* in vitro, a culture of the organisms was spread on the surface of Kulp's tomato juice agar medium (pH 5), and three wells were cut in the agar plate. Into two of the wells 0.2 c.c. of 0.5 molar sodium bicarbonate and sodium hydroxide solutions were introduced, respectively. Into the third well 0.5 molar sodium carbonate was introduced, the same volume, 0.2 c.c., being employed. After incubating for twenty-four hours, the plate was examined and it was

observed that there was an area around the rim of each well which was free of organisms. The diameter of the wells was 8.7 millimeters. The diameter of the well plus the clear area around it was as follows:

Sodium bicarbonate	19.4 mm.
Sodium hydroxide	17.5 mm.
Sodium carbonate	25.0 mm.

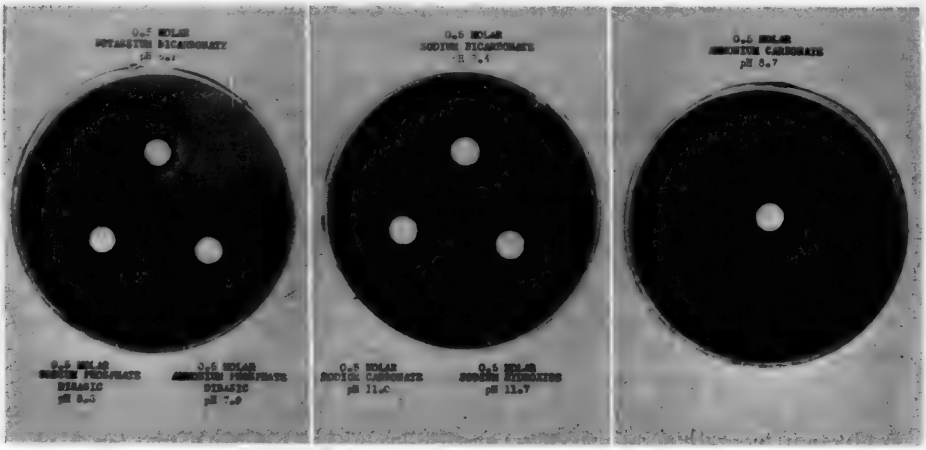
As a matter of interest, other alkalis were tested similarly, all solutions being 0.5 molar. The diameter of the wells was 8.4 mm., except the well containing ammonium carbonate which had a diameter of 8.8 millimeters.

The diameter of well plus clear area was as follows:

Potassium bicarbonate	17.5 mm.
Ammonium phosphate (dibasic)	8.4 mm.
Sodium phosphate (dibasic)	8.4 mm.
Ammonium carbonate	21.0 mm.

DISCUSSION

It is difficult to ascertain to what extent pH, diffusion of the solutions through the agar, and any specific inhibitory effect of a given solution, prevent bacterial growth. Perhaps greatest weight should be placed on the pH changes that occur.



A. *B.* *C.*

Fig. 2.—Photograph of areas of growth inhibition around wells in agar plates filled with alkaline reagents as indicated. The organism is type II *L. acidophilus*.

In the photographs of these plates (Fig. 2) the pH of the respective solutions is indicated, but this pH is probably altered when it comes into contact with the agar medium. The pH is again altered, especially in the case of the carbonates, during incubation. This was ascertained by determining pH changes that occurred in incubated sterile broth to which 0.2 c.c. of 0.5 molar solutions

of the previously mentioned reagents had been added. The findings are briefly tabulated as follows:

0.5 M SOLUTION	PH OF ORIGINAL SOLUTION	PH OF BROTH PLUS SOLUTION BEFORE INCUBATION	PH OF BROTH PLUS SOLUTION AFTER INCUBATION
Sod. bicarbonate	8.4	6.4	7.2
Sod. carbonate	11.0	7.5	8.0
Sod. hydroxide	11.7	7.6	7.1
Amm. carbonate	8.7	7.5	7.9
Amm. phosphate, dibasic	7.9	6.6	6.5
Sod. phosphate, dibasic	8.8	6.7	6.6

While this procedure does not precisely duplicate the conditions that prevail in the wells, it indicates the nature of the pH changes. It will be noted that the carbonate-adjusted broth tends to rise in pH during incubation, whereas the other reagents remain at approximately the same pH levels with a tendency to decrease.

In extending these findings to the oral cavity, it is interesting to note that in eighteen individuals studied, the *L. acidophilus* counts showed a general trend downward at the end of two months during which sodium bicarbonate was used as a dentifrice. The acidophilus counts were reduced or maintained at zero over a period of six months in a group of nine individuals similarly studied.

It may be concluded from these experiments that sodium bicarbonate inhibits the growth of *L. acidophilus* in vitro, and there appears to be a correlation between this inhibition and the changes in alkalinity that occur in the course of incubation.

SUMMARY

Adherent, cloudy films of plaque-like nature have been deposited on sheets of cellophane and their effects on the rate of dialysis of sodium bicarbonate through the cellophane have been studied. The plaques slightly inhibit but do not prevent the passage of the bicarbonate through the membrane.

The effect of sodium bicarbonate on acid production and growth of *L. acidophilus* has been studied in vitro. Inhibition of growth occurs which, it is believed, can be attributed in great measure to pH changes that occur in the media. However, reference is made to a limited number of individuals whose *L. acidophilus* count was reduced or maintained negative during two- and six-month periods in which sodium bicarbonate was employed as a dentifrice.

The investigator wishes to acknowledge his indebtedness to Dr. Thomas J. Hill whose advice and guidance were so generously proffered throughout the investigation.

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THE ROLE OF OXALATES ON THE INCIDENCE AND EXTENT OF DENTAL CARIES IN THE COTTON RAT (SIGMODON HISPIDUS HISPIDUS)

S. R. HOWELL, D.M.D., C. A. SCHLACK, D.D.S., B. L. TAYLOR, AND V. J. BERZINSKAS

INTRODUCTION

OUR previous studies with oxalates in relation to acid beverages[1] led us to explore the possible role of dietary oxalates in connection with tooth decay. Several experiments were performed using white rats on a coarse corn meal diet with varying amounts of oxalates in the food and water. Our data indicated that oxalates had no effect on dental caries in these animals.[2]

Through the work of Shaw, Schweigert, Elvehjem, and their collaborators,[3-10] our attention was turned to the use of the cotton rat for similar studies. White rats are susceptible to caries on a coarse corn meal diet, whereas cotton rats are susceptible to caries on a sugar diet. It was felt that by using the cotton rat the species and dietary differences would help substantiate the results already obtained from the use of oxalates in the diet of the white rat.

PROCEDURE

Fifty-one weanling litter mate cotton rats (Group I) were placed on a sugar caries-producing diet with the addition of 4 per cent liver extract employed by Shaw, Schweigert, McIntire, Elvehjem, and Phillips.[4] They were divided into three equal subgroups (A, B, and C) in such a manner that a rat in subgroup A had a litter mate in subgroups B and C. Subgroup A (control) drank only distilled water, and subgroups B and C received, respectively, 0.02 per cent and 0.22 per cent potassium oxalate in distilled water to drink ad libitum. This dietary was maintained for a period of fourteen weeks which is the time required to produce the desired degree of dental caries.

During the course of this experiment twenty-one of the fifty-one rats died. Post-mortem examination revealed hair balls in the stomach (trichobezoars) as the probable cause of death. Following a suggestion by McCay,[11] 10 per cent by weight of cellulose was then added to the caries-producing diet. This prevented the formation of hair balls and eliminated the mortality.

Group II, consisting of fifteen rats, received the same basic caries-producing diet but with the 10 per cent by weight of cellulose added. These rats were similarly divided into three equal subgroups (A, B, and C) of five rats each. Subgroup A (control) drank only distilled water, subgroup B drank distilled water with the addition of .0002 per cent potassium oxalate, and subgroup C drank distilled water with the addition of .002 per cent potassium oxalate. These animals likewise were kept on this diet for a period of fourteen weeks.

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All sixty-six rats were kept under identical environmental conditions, the temperature and humidity of the animal laboratory being constant throughout the experimental period.

At the end of the experiment all surviving rats were weighed and autopsied. The jaws were prepared and examined for dental caries in a manner described by a previous report.[12]

The results of the caries examination were tabulated, but only those litter mates of Group I that survived the entire fourteen weeks were used for evaluation. Using these data, an analysis of variance[13, 14] was made within groups I and II to show the effect of oxalates on caries incidence and extent, and weight gains. Similarly, an analysis[15] was made to assess any effect of the cellulose on dental caries incidence and extent.

RESULTS

Of the fifty-one rats in Group I only thirty lived the full fourteen weeks, and of these thirty only six control rats (subgroup A) had surviving litter mates in the other two subgroups (B and C), leaving a total of eighteen rats for evaluation of caries incidence and extent (Table I). All fifteen rats of Group II lived the entire fourteen weeks remaining healthy and showing substantial weight gains (Table I).

In Table I, subgroups A, B, and C of Group I showed a mean caries incidence varying between 14.8 and 20.8 with a mean caries extent between a total score of 24.8 and 39.7. The mean weight gain of these subgroups ranged between 44.7 and 64.3 grams. Subgroups A, B, and C of Group II showed a mean caries incidence ranging between 14.0 and 23.8 with a mean caries extent between a total score of 25.8 and 66.4. The mean weight gain of these subgroups ranged between 79.0 and 82.4 grams.

By applying an analysis of variance (Table II) to the data shown in Table I it may be seen that no significant difference could be found in caries incidence and extent or weights gained, indicating a lack of influence of the oxalates in the drinking water on either Group I or Group II.

To determine whether or not the cellulose had any effect on the incidence or extent of dental caries an analysis between subgroup A of Group I and subgroup A of Group II was made in a manner outlined in a previous report.[2] Subgroups A of Groups I and II were comparable except for dietary cellulose in subgroup II A. From the analysis of the data (Table III) it is evident that there was no significant difference in the incidence and extent of dental caries. This would seem to indicate that the cellulose played no role in the caries picture.

DISCUSSION

Our dental caries experiments with white rats on a corn meal diet[2] showed that the oxalates apparently played no role in the incidence and extent of carious lesions. It was felt that these results might have been typical only of that species of rat on that particular type of caries-producing diet. This led to the use of the cotton rat which developed carious lesions on a synthetic diet consisting chiefly of sugar. Here again, however, oxalates seem to have no influence on the incidence and extent of caries. Since no significant differences

TABLE I

DENTAL CARIES INCIDENCE AND EXTENT, AND WEIGHT GAINS IN COTTON RATS ON CARIES-PRODUCING DIETS RECEIVING DRINKING WATER CONTAINING VARYING AMOUNTS OF POTASSIUM OXALATE. AGE—THREE WEEKS; DURATION OF DIET—FOURTEEN WEEKS

Group I—Basic Diet, Caries-producing

	A: CARIES DIET + DISTILLED DRINKING WATER (CONTROL)							B: CARIES DIET + .02% POTASSIUM OXALATE IN DRINKING WATER							C: CARIES DIET + .22% POTASSIUM OXALATE IN DRINKING WATER						
	RAT NO.	LITTER NO.	SEX	CARIES INCID.	TOTAL EXTENT	AVER. EXT.	WEIGHT GAIN GM.	RAT NO.	LITTER NO.	SEX	CARIES INCID.	TOTAL EXTENT	AVER. EXT.	WEIGHT GAIN GM.	RAT NO.	LITTER NO.	SEX	CARIES INCID.	TOTAL EXTENT	AVER. EXT.	WEIGHT GAIN GM.
	69	C-30	F	25	45	1.8	33	70	C-30	M	21	43	2.04	56	72	C-30	M	15	32	2.1	51
	74	C-31	M	15	18	1.2	42	76	C-31	M	12	22	1.8	41	78	C-31	M	17	30	1.7	48
	86	C-33	M	20	35	1.7	78	88	C-33	M	14	21	1.5	78	90	C-33	M	19	45	2.3	52
	104	C-37	M	14	30	2.1	49	106	C-37	M	10	13	1.5	71	108	C-37	M	15	30	2.0	59
	105	C-38	F	34	60	1.8	24	107	C-38	F	17	23	1.3	82	109	C-38	F	29	67	2.3	24
	116	C-41	F	17	40	2.3	42	117	C-41	M	15	27	1.8	58	118	C-41	F	14	34	2.4	41
Total	6	6		125	228	1.8	268	6	6		89	149	1.7	386	6	6		109	238	2.2	275
Mean				20.8	38.0	1.8	44.7				14.8	24.8	1.7	64.3				18.1	39.7	2.2	45.9
S.D.σ				7.574	14.213		18.480				3.969	10.008		15.469				5.590	14.501		12.175
E.M.$\sigma\chi$				3.387	6.356		8.265				1.730	4.475		6.918				2.500	6.485		5.445

Group II—Basic Diet, Caries-producing + 10% Cellulose

	A: CARIES DIET + DISTILLED DRINKING WATER (CONTROL)							B: CARIES DIET + .0002% POTASSIUM OXALATE IN DRINKING WATER							C: CARIES DIET + .002% POTASSIUM OXALATE IN DRINKING WATER						
	119	C-42	M	11	22	2.0	78	121	C-42	M	22	53	2.4	90	123	C-42	F	34	132	3.8	81
	120	C-44	F	17	36	2.1	71	122	C-44	F	30	93	3.1	40	124	C-44	M	30	87	2.9	63
	125	C-43	M	19	33	1.7	104	127	C-43	M	13	20	1.5	86	129	C-43	M	20	35	1.7	76
	126	C-43	F	11	19	1.7	81	128	C-43	F	12	20	1.6	88	130	C-43	M	22	59	2.1	93
	131	C-45	M	12	19	1.5	78	132	C-45	M	10	16	1.6	91	133	C-45	M	13	19	1.4	83
Total	5	4		70	129	1.8	412	5	4		87	202	2.3	395	5	4		119	332	2.8	396
Mean				14	25.8	1.8	82.4				17.4	40.4	2.3	79.0				23.8	66.4	2.8	79.2
S.D.σ				4.243	8.097		12.618				8.562	32.990		21.286				8.319	44.764		10.054
E.M.$\sigma\chi$				2.122	4.048		6.308				4.281	16.495		10.943				4.129	11.191		5.027

TABLE II

ANALYSIS OF VARIANCE FOR CARIES INCIDENCE AND EXTENT, AND WEIGHT GAINS IN COTTON RATS ON A CARIES DIET WITH VARYING AMOUNTS OF OXALATE IN THE DRINKING WATER

Group I

INCIDENCE						EXTENT						WEIGHT GAINS					
SOURCE OF VARIANCE	SUM OF MEAN SQUARES	DEGREES OF FREEDOM	MEAN VARIANCE	RATIO OF ERROR TO DIET AND LITTER	F = .01	SOURCE OF VARIANCE	SUM OF MEAN SQUARES	DEGREES OF FREEDOM	MEAN VARIANCE	RATIO OF ERROR TO DIET AND LITTER	F = .01	SOURCE OF VARIANCE	SUM OF MEAN SQUARES	DEGREES OF FREEDOM	MEAN VARIANCE	RATIO OF ERROR TO DIET AND LITTER	F = .01
Diet and water oxalate	108	2	54	3.94	7.56	Diet and water oxalate	792	2	396	3.71	7.56	Diet and water oxalate	1461	2	730.5	3.69	7.56 5.64
Litter	372	5	74.4	5.43	5.64	Litter	1497	5	299.4	2.81	5.64	Litter	1669	5	333.8	1.69	
Error (LxD)	137	10	13.7			Error (LxD)	1067	10	106.7			Error (LxD)	1978	10	197.8		
Total	617	17				Total	3356	17				Total	5108	17			
Ratio for diets = 3.94 P > .01 Ratio for litters = 5.43 P > .01						Ratio for diets = 3.71 P > .01 Ratio for litters = 2.81 P > .01						Ratio for diets = 3.69 P > .01 Ratio for litters = 1.69 P > .01					

Group II

INCIDENCE						EXTENT						WEIGHT GAINS					
Diet and water oxalate	231	2	115.5	3.73	10.92	Diet and water oxalate	4164	2	2032	2.63	10.92	Diet and water oxalate	19	2	9.5	0.08	10.92
Litter	349	3	116.3	3.75	9.78	Litter	6615	3	2203	2.85	9.78	Litter	1702	3	567	4.88	9.78
Error (LxD)	186	6	31.0			Error (LxD)	4630	6	774			Error (LxD)	698	6	116		
Total	766	11				Total	15419	11				Total	2419	11			
Ratio for diets = 3.73 P > .01 Ratio for litters = 3.75 P > .01						Ratio for diets = 2.63 P > .01 Ratio for litters = 2.85 P > .01						Ratio for diets = .08 P > .01 Ratio for litters = 4.88 P > .01					

No significant difference between caries incidence and extent or weight gains of cotton rats on different oxalate-containing dietaries.

TABLE III

STATISTICAL ANALYSIS BETWEEN CONTROL SUBGROUPS IA AND IIA TO DETERMINE EFFECT OF CELLULOSE ON CARIES INCIDENCE AND EXTENT

GROUPS	NUMBER OF RATS	DENTAL CARIES INCIDENCE			DENTAL CARIES EXTENT		
		MEAN	STANDARD DEVIATION	ERROR OF THE MEAN	MEAN	STANDARD DEVIATION	ERROR OF THE MEAN
IA (No cellulose)	6	20.8	7.574	3.387	38.0	14.213	6.356
IIA (10% cellulose)	5	14.0	4.243	2.122	25.8	8.097	4.048

	GROUPS ANALYZED	STANDARD DEVIATION OF THE DIFFERENCE	DIFFERENCE/ ERROR	DEGREES OF FREEDOM	T = 1% LEVEL	T = 5% LEVEL	
Dental caries incidence	IA and IIA	3.74	1.818	9	3.250	2.262	P > .01 or .05 = No significant difference
Dental caries extent	IA and IIA	7.2	1.694	9	3.250	2.262	P > .01 or .05 = No significant difference

In dental caries incidence and extent, there was no significant difference between rats on diets with or without cellulose.

were found in the incidence and extent of dental caries between the groups receiving oxalates and those receiving no oxalates it may be assumed that either there was no real difference or the groups used were too small to reveal a difference, if one existed.

Cellulose apparently does not influence the development of caries, but this substance evidently is required by the cotton rat as inert dietary material.[11]

There was some question as to whether the oxalates added to the water had a detrimental effect[16] on the cotton rat or had been a contributing factor in the premature deaths in Group I. As may be seen in Tables I and II, however, it appears that the mortality rate of the control rats was as high as those receiving oxalates in Group I.

SUMMARY AND CONCLUSIONS

1. Varying amounts of potassium oxalate in the drinking water of cotton rats on a caries-producing synthetic diet apparently had no effect on the incidence or extent of carious lesions.

2. Cellulose added to the diet for the prevention of trichobezoar formation apparently did not influence caries incidence or extent.

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SODIUM FLUORIDE REAGENT CONCENTRATION AND POWDERED ENAMEL SOLUBILITY

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INTRODUCTION

ONE of the phases of tooth decay, that is, cavitation, for a long time has been assumed to be a dissolution of the tooth mineral material by a weak organic acid, presumably lactic acid. Although direct experimental proof of the assumption is lacking, it is difficult to see at present how it could be otherwise. The effect of fluorine in caries inhibition in human beings in the clinic and albino rats in the laboratory has added to, rather than detracted from, this assumption. This is particularly true since laboratory studies have demonstrated that the mechanism of the effectiveness of fluorides may be through change in solubility of the tissues involved in the disease.[1-4]

The effect of fluorine upon the solubility of calcium phosphates had been studied previous to the time when fluorine first became recognized as an inhibitor of tooth decay.[5] On the basis of work carried out with respect to the effect of fluorine on phosphate and bone solubility, it was even predicted that fluorine might reduce the solubility of dental tissues. Volker[6] first demonstrated that fluorine did affect tooth tissue solubility. Buonocore and Bibby[7] demonstrated that certain reagents other than sodium fluoride reduced enamel solubility and that the effect varied with different reagents. This point was further emphasized by work done by Muhler and Van Huysen.[8]

As to the optimum concentration of the fluoride solutions used clinically in the inhibition of tooth decay and in the laboratory studies of enamel solubility, there is no unanimity of opinion. Most clinical investigators use one or two per cent solutions of sodium fluoride and believe that the latter is about as high a concentration as is safe to use with topical methods of application. In most of the in vitro studies of enamel solubility, a concentration of 1/500 reagent solution has been used.

In addition to the question of fluoride concentration of reagent solutions, there is also the problem of correct pH of these protecting reagent solutions. It was Bibby[9] who first pointed out that acidulated reagent solutions were more effective in reducing enamel solubility than were those at, or around, a neutral pH. In his measurements of the effectiveness of various enamel solubility reducing reagents, he used a pH of 4.0.

It is proposed that in this study the effectiveness in reducing enamel solubility of various reagent solution concentrations be examined. Since sodium fluoride is the reagent solution most commonly used to inhibit tooth decay and

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to reduce powdered enamel solubility in laboratory experiments, it was decided that this reagent, buffered and unbuffered, be used in this study.

PROCEDURE

Preparation of Reagent Solutions.—The first step in this study was the preparation of nine buffered and unbuffered sodium fluoride reagent solutions at concentrations of 0.2, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 per cent (Table I). These reagents were made by placing the calculated amounts of sodium fluoride in nine flasks and adding sufficient water to make a total volume of 500 millimeters. After the pH of each of these solutions was measured, the nine 500 ml. solutions were divided equally and one 250 ml. portion in each instance had added to it 5 ml. of HAC. The 250 ml. acidulated portions were used as the buffered solutions; the untreated 250 ml. portions were used, of course, as the unbuffered solutions. The sodium fluoride was Merck's reagent quality.

I. Enamel Solubility Standard.—This study is arranged to show the degree with which reagent solutions (sodium fluoride) of different concentration and pH protect or reduce the solubility of tooth enamel. The basis for such a comparison is the average solubility of the sample of powdered tooth enamel used in the study. This average for comparison was obtained by determining the solubility of 0.1000 Gm. of the powdered enamel in 25 ml. of acetate buffer adjusted to a pH of 4.0.

A variety of the Royal Berlin crucible known as the Selas filtering crucible was used to contain the acid and powdered enamel. The pore size of the filter beds of the crucibles used was estimated to be about seven microns. The crucibles placed in a porcelain dish for insulation were heated over a Meker burner to establish a constant weight and were stored in a desiccator until ready for use. Into each tared crucible there was weighed 0.1000 Gm. of powdered enamel that was prepared by the method of Manly and Hodge.[10] A uniform particle size was obtained in a manner described in a previous paper.[9] This enamel was then treated with 25 ml. of the 0.2M acetate buffer prepared from glacial acetic acid and adjusted to a pH 4.0 with IN NaOH. The contents of each crucible were stirred for forty minutes at room temperature. Any remaining debris was removed from the stirring rod by a few milliliters (constant volume in each instance) of distilled water. Each crucible was taken to a suction filter and the liquid phase removed. The crucible contents were then washed with a few milliliters of distilled water (constant volume) and the washings removed by suction.* The crucibles were then dried at 70° C. for three hours, placed in a desiccator for six hours, and reweighed. A similar procedure for drying and desiccating was followed until no greater variance than 0.0001—0.0002 Gm. was encountered in each crucible. This procedure was repeated with additional crucibles until data from twelve such tests were available. This part of the procedure tested the solubility of powdered enamel before treatment

*Special precautions were taken in an attempt to rinse all crucibles with the same amount of distilled water, for distilled water has the ability to decalcify the enamel proper. When done carefully, however, this has only an infinitesimal effect on the procedure, and if held constant in each case, may thus be eliminated as a source of error.

with sodium fluoride, and data were used as a basis with which to compare the solubility of the protected enamel. It was found that there was a loss of 0.0280 Gm. in each of the 0.1000 Gm. of the powdered enamel decalcified.* This average loss of weight of unprotected enamel is shown in line 1, column 3 of Table I, and is the basis with which the remaining values are compared; that is, those for the solubility of the reagent-protected enamel. These remaining values make up the bulk of Table I and are determined by the procedure outlined in the next section of the paper.

II. Solubility of Enamel Treated With Different Concentrations of Buffered and Unbuffered Sodium Fluoride.—The effect the various concentrations of reagent solutions had in inhibiting the solubility of powdered enamel was determined by weighing again into each of several dried and tared crucibles 0.1000 Gm. of this tissue. Each of these enamel portions was then treated with 5 ml. of the sodium fluoride reagent solution ranging in concentration from 0.2 to 4.0 per cent (Table I). In each case, the enamel and reagent solutions were stirred for twenty minutes and any remaining debris was removed from the stirring rod with a few milliliters (constant volume) of distilled water. They were filtered by means of an aspirator, washed with distilled water, and dried as described. This same procedure was used with both the unbuffered and buffered solutions.

In each instance 20 ml. of the decalcifying solution were added to the treated enamel and the combinations were stirred for twenty additional minutes. They were filtered, washed, dried, desiccated, and reweighed in the manner described. In each case several crucibles were treated in a like manner, with the same precautions followed for legitimate data as mentioned previously. In each instance the data in the table include an average of twelve tests.

DATA

Table I shows in the first row of the first three columns the weight change brought about by acetic acid when combined with unprotected enamel. In all other instances the data show weight loss obtained when the acid is combined with protected enamel. The enamel was protected by buffered and unbuffered solutions of sodium fluoride reagent. Column 1 of Table I lists the varying concentrations of reagent solutions. The first row of columns 2 and 3 gives the pH and weight loss, respectively, that was brought about when unprotected enamel samples were used. Column 2 shows the pH of these solutions prior to adjustment.† Column 3 shows in the first row the loss in weight that took place in the 0.1000 Gm. sample of enamel that was unprotected. The bulk of column 3 contains data to show loss in weight of enamel that was protected with a solution of unbuffered sodium fluoride at the respective concentrations. Column 4 shows the pH of the acidulated reagents, i.e., the pH after 5 ml. HAC

*From reading the literature it is apparent that weight loss and actual decalcification of the enamel are assumed to be the same process. While this may be the same situation in many instances, until it is definitely proved that there is a loss of Ca and P from the tissues during combination with the reagents used, the terms should be more adequately defined. In this paper, weight loss means the weight change from the original sample and not necessarily decalcification.

†Adjustment simply refers to the process whereby HAC was added to the sodium fluoride solution.

TABLE I

SHOWING HOW CONCENTRATION OF SODIUM FLUORIDE UNBUFFERED AND BUFFERED AT VARYING LEVELS OF pH AFFECTS SOLUBILITY OF POWDERED ENAMEL

REAGENT SOLUTION CONCENTRATION	UNBUFFERED		BUFFERED	
	REAGENT SOLUTION pH	WEIGHT CHANGE (GRAMS)	REAGENT SOLUTION pH	WEIGHT CHANGE (GRAMS)
0.2M HAC	4.00	−0.0280		
0.2%	6.10	−0.0134	3.70	−0.0116
0.5%	6.30	−0.0120	3.90	−0.0072
1.0%	6.60	−0.0115	4.10	−0.0076
1.5%	6.75	−0.0146	4.35	−0.0016
2.0%	6.90	−0.0115	4.50	−0.0001
2.5%	7.00	−0.0108	4.53	−0.0053
3.0%	7.05	−0.0110	4.70	−0.0078
3.5%	7.00	−0.0112	4.60	−0.0029
4.0%	7.25	−0.0112	4.90	−0.0068

had been added to one-half of the original sodium fluoride solutions to provide the buffered reagents. Column 5 lists the weight changes brought about in the enamel that was protected with 5 ml. of the buffered sodium fluoride reagent solutions. In addition to Table I these data are also arranged in Fig. 1.

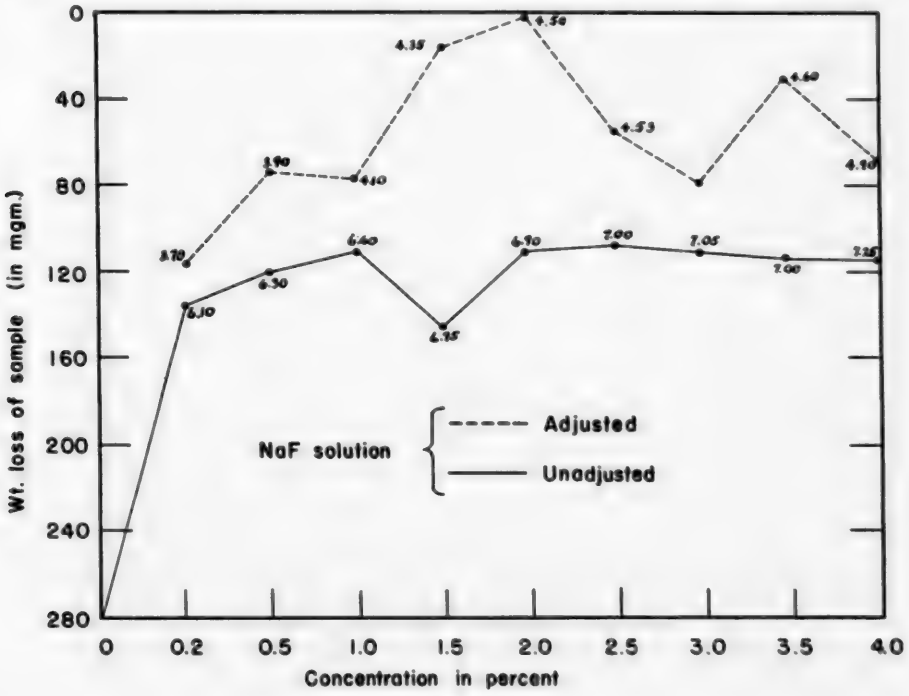

Fig. 1.

As the concentrations were increased, i.e., from 0.2 to 4.0 per cent, there was, of course, an increase in the weight of the sodium fluoride in solution. In order to measure the true effect the reagent solution had in this process, a

similar amount of the respective reagent solution was placed in a crucible and filtered, washed, dried, and desiccated in the manner described previously. These crucibles were weighed as controls, and the residual weights were subtracted from the experimental values before being recorded in the table.

In an attempt to prepare a 4 per cent solution of sodium fluoride buffered to a pH 4.0, it was discovered that it took so much acetic acid that the protective effect of the fluorine was lost and the hydrogen fluoride reaction was very much exaggerated. Letting HAC represent acetic acid, the reaction would proceed in the following manner.

$$HAC + NaF \rightleftharpoons Na^+ Ac^- + H^+ F^-$$

The HF produced a marked etching on the inside of the glass bottles that were used to store the reagent solutions. In fact, only the solutions above 2.5 per cent showed evidence of bottle etching although all bottles contained the same amount of acetic acid. Naturally, the concentration of NaF was different in each case. Because of this phenomenon, it was of interest to know what effect the products of this reaction had in inhibiting the dissolution of the enamel. Table II shows the effect sodium acetate has in inhibiting the dissolution.

TABLE II

SHOWS WEIGHT CHANGE OF ENAMEL AFTER PROTECTION WITH NaAc OF VARYING pH'S AND CONCENTRATIONS AFTER COMBINATION WITH A DECALCIFYING SOLUTION

	UNBUFFERED		BUFFERED	
REAGENT SOLUTION CONCENTRATION	REAGENT SOLUTION pH	WEIGHT CHANGE (GRAMS)	REAGENT SOLUTION pH	WEIGHT CHANGE (GRAMS)
0.2M HAC	4.00	−0.0280		
0.2%	7.50	−0.0192	3.60	−0.0256
0.5%	7.46	−0.0169	3.40	−0.0259
1.0%	7.55	−0.0185	3.71	−0.0246
1.5%	7.60	−0.0196	3.95	−0.0231
2.0%	7.70	−0.0177	4.12	−0.0219
2.5%	7.70	−0.0143	4.20	−0.0222
3.0%	7.80	−0.0145	4.40	−0.0230
3.5%	7.88	−0.0189	4.32	−0.0235
4.0%	7.90	−0.0170	4.30	−0.0226

Table II corresponds to Table I, only the reagent in this case is NaAc in place of NaF. Column 1 of Table II lists the concentration of the different sodium acetate solutions. Column 2 lists the pH prior to addition of acetic acid. Column 3 shows the weight changes of the enamel after protection with 5 ml. of this unbuffered reagent solution. Column 4 shows the pH of the sodium acetate solution after adding 5 ml. of glacial acetic acid. Column 5 lists the protection this buffered solution affords the powdered enamel.

DISCUSSION

It is not definitely known how the fluoride ion enters the apatite molecule; therefore, one must speculate, in most cases, with respect to this phenomenon.

One would suppose, from the laws of combining proportions and rules of solubility, that with a rise in concentration* of the reagent solution used to protect the powdered enamel there would be a corresponding decrease in the enamel solubility provided that both compounds were soluble to an appreciable extent.

Table I shows that when an unbuffered solution of sodium fluoride is used there is a definite decrease in solubility of the enamel protected by it. There is a reduction in solubility ranging from 14.6 mg. for a 0.2 per cent solution to 16.8 mg. for a 4.0 per cent solution. When a buffered solution of sodium fluoride is used to protect the enamel this changes to 16.4 mg. for a 0.2 per cent solution to 21.2 mg. for a 4.0 per cent solution. The greatest solubility reduction is found at a concentration of 2.5 per cent. In the buffered solution the greatest protecting effect is reached at a concentration of 2.0 per cent (Fig. 1).

One can quickly see from Fig. 1 that in contrast the buffered solution is better than the unbuffered solution for protecting the enamel, regardless of what concentration of sodium fluoride one may choose. It reaches its maximum protecting effect at a concentration of 2.0 per cent. The pH at this concentration is 4.5. Why the enamel protection is increased when the concentration changes from 3.0 to 3.5 per cent cannot at this time be explained. It is believed that at this concentration and pH the reaction between this amount of sodium fluoride and acetic acid is more highly dissociated. This is illustrated in the foregoing formula. Fig. 1 also shows that there is no correlation between the effectiveness of the buffered and unbuffered solutions.

Only a word need be mentioned with respect to the sodium acetate and its effect upon solubility. It was only logical to suppose in certain instances (irrespective of equilibrium) that in a solution composed of acetic acid and sodium fluoride, there would be sodium ions, fluorine ions, hydrogen ions, and the acetate ions, while under different conditions there would be various but definite combinations of these ions. Therefore, one of the products of this reaction is sodium acetate. In testing the ability of sodium acetate to decrease the solubility of powdered enamel, it was discovered that there was no correlation between the buffered or unbuffered solutions, nor any reasonable protection offered the enamel by any concentration of this reagent. Hydrogen fluoride was not tested because of the extreme difficulties associated with its storage and reactivity. This does not mean that it has been eliminated as a possible enamel-protecting reagent.

One would expect to find the maximum protection in this experiment when a 4 per cent solution is used. This is not the case by any means. The maximum protection when the buffered reagent solution is used is found when the solution concentration is 2 per cent. There is a marked decrease in effectiveness from 2 to 3 per cent and an increase with 3.5 per cent, with a further decrease at 4 per cent. Various phenomena could be operating to produce this effect.

*Concentration used here is the measure of the number of molecules of a reacting substance in a given unit-volume and not necessarily the total quantity of a substance which is present. The speed of a reaction is proportional to the molecular concentration of each of the substances which take part in the reaction. This effect is described as the law of mass action. Concentration refers in this case to the number of gram-molecular weights of each reacting substance per liter.

Some were postulated in a previous paper.[8] For the present, it must be concluded that much fundamental knowledge is yet to be had concerning the products formed by this reaction, their ionization constants, chemical equilibrium, and concentration.

The amount of substance present and capable of reacting is a very important factor influencing reacting velocity.[12] The effect of the concentration of the reacting components on the speed of the reaction, called mass action, is of fundamental importance in the study of these in vitro chemical changes. The amount of substance transformed in unit time depends upon the amount of each reactant present, temperature, and other factors remaining constant. Doubling the amount of one reactant will necessarily result in more being transformed into the products of the reaction, but this does not necessarily imply that it is capable of reducing enamel solubility.

CONCLUSIONS

1. In this experiment a 2 per cent solution of sodium fluoride is found to be the best concentration to use for reducing the solubility of powdered enamel.

2. Buffered solutions of sodium fluoride are much more effective than unbuffered solutions.

3. The most effective pH for a 2 per cent reagent solution is 4.5.

4. It is suggested that at a concentration of 2 and 3.5 per cent, respectively, the solution is more highly dissociated than at any other concentration used, thus making it more effective in its ability to reduce "enamel solubility."

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A MEDIUM FOR THE DIFFERENTIATION OF STRONGLY ACIDOGENIC FROM WEAKLY ACIDOGENIC ORGANISMS OF HUMAN SALIVA

A Preliminary Report

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EVER since Miller propounded the chemico-parasitic theory of dental caries, numerous endeavors have been made to isolate an organism or group of organisms as the specific etiological factor.[1-15] Recent studies emphasize the importance of the rate at which acid is formed[16, 17] and the total titratable acidity produced[18] regardless of the organism responsible for the carbohydrate degradation. From in vitro experiments it has been shown[19, 20] that different strains of lactobacilli, streptococci, and staphylococci exhibit varying acidogenic capacities, and that it is virtually impossible to predict the potential acidogenic capacity of any given strain from the colony morphology or cellular appearance.

Of the many bacteriological tests for caries activity the two most widely used depend on (1) the estimation of the number of colonies of lactobacilli growing out in four days on a tomato agar plate of pH 5.0 from an inoculum of saliva,[21] and (2) the colorimetric estimation of the acid-producing capacity of the saliva by inoculating 0.1-0.2 ml. of it into melted infusion agar containing 2 per cent dextrose; the initial pH is adjusted to 5.0 and the indicator is bromcresol green.[22] The latter test is a guide to the acid potential of saliva and is a simple test to carry out. It is, however, a purely qualitative test and gives no indication as to the relative numbers of acidogenic and nonacidogenic organisms present in a given sample of saliva. This same criticism is applicable to the various glucose broth tests. The tomato agar plate method is an index of the numbers of aerobic *aciduric* organisms present in saliva, but it is not necessarily an accurate guide to the number of strongly acidogenic organisms present.

The present study is concerned with the quantitative differentiation of strongly acidogenic from weakly acidogenic components of the oral flora.

EXPERIMENTAL

Procedure:

The medium used is a modification of that devised by Wade, Smiley, and Boruff[23, 24] for estimating the numbers of acidogenic bacteria in distillery mashes.

Tomato juice (filtered)	300 ml.
Yeast extract	5 Gm.
Glucose	5 Gm.
Salts "A"*	5 ml.
Agar	30 Gm.
1.6% alcoholic solution bromcresol purple	2 ml.
Distilled water to	1,000 ml.

*Salts "A":—25 Gm. each of monobasic and dibasic potassium phosphate in 100 ml. of distilled water.

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The pH is adjusted to 7.0. The medium is dispensed in 100 ml. amounts, autoclaved, and stored in a refrigerator.

Saliva, stimulated by the chewing of paraffin, is collected before breakfast on the morning of the test. Dilutions are made up as follows: Samples are collected and shaken vigorously. One milliliter is pipetted into 99 ml. of nutrient broth to give a dilution of 1:100. This is shaken well and 1.0 ml. is pipetted into a second flask of 99 ml. of nutrient broth to give a dilution of 1:10,000. For these preliminary experiments 0.1 ml. of the 1:10,000 dilution is used.

Before each experiment the medium is reheated in a water bath until fluid. Five milliliters of 8 per cent dibasic calcium phosphate in water suspension is then added to each 100 ml. of medium and mixed with the medium by rotation of the flask. When the temperature falls to approximately 45° C., the medium is rotated vigorously and poured in 20 ml. amounts into petri dishes previously inoculated with 0.1 ml. of the test saliva dilution. Each plate is rotated quickly to disperse the inoculum through the medium. The agar will set sufficiently quickly at room temperature to prevent settling out of the phosphate suspension.

All plates are incubated at 37° C. for four days.

Distinguishing the Colonies:

1. ***Strongly acidogenic colonies*** dissolve the calcium phosphate, forming a large halo of 3 mm. or more in diameter, and change the color of the indicator from purple to yellow (Fig. 1).

2. ***Moderately acidogenic colonies*** give rise to small halos much more limited in extent than those in (1) and easily distinguishable therefrom. They also change the indicator from purple to yellow (Fig. 3).

3. ***Weakly acidogenic colonies*** change the color of the indicator but do not dissolve the calcium phosphate.

4. ***Neutral colonies*** produce no change.

5. ***Alkali-producing colonies*** show a peripheral zone of deep purple color. They do not form halos.

RESULTS

The medium was first tested by means of cultures of known properties (lactobacilli, streptococci, and staphylococci). It was found that various strains of lactobacilli and streptococci could be distinguished, visually, by their acidogenic capacity when grown in this medium. Organisms producing clearing were subcultured and stained by Gram's method. Of twenty-two organisms producing large zones of clearing, fourteen were formed by a lactobacillus "S" (Fig. 2), three by a lactobacillus "R," and five by a streptococcus. Of thirty-eight organisms producing small zones of clearing, twenty-seven were formed by a streptococcus (Fig. 4), three by a lactobacillus "R," two by a diplococcus, and six by pleomorphic organisms which could not be differentiated by their cellular appearance. The appearance of each was similar to that described by Rosebury[25] and classified as indefinite—either a streptococcus undergoing a pleomorphic change to resemble a lactobacillus, or a lactobacillus undergoing a change to resemble a streptococcus. The organisms producing alkali were found to be spore-forming rods or molds.

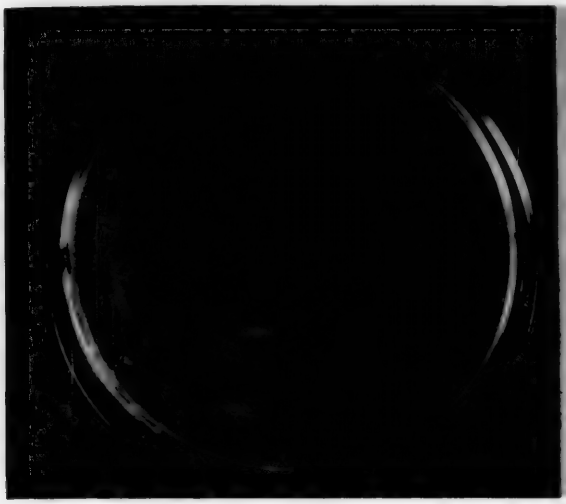

Fig. 1.

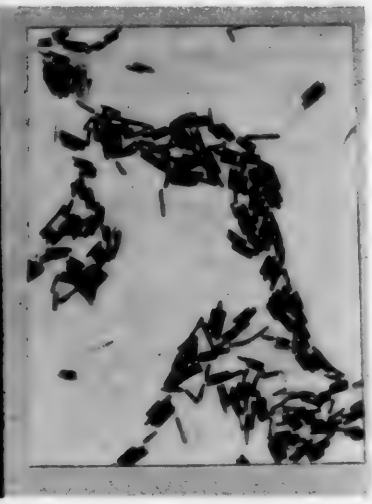

Fig. 2.

Fig. 1.—Large zone of clearing as produced by Lactobacillus, "S" form; ×0.65.
Fig. 2.—Lactobacillus from colony in Fig. 1, stained by Gram's method; ×1100.

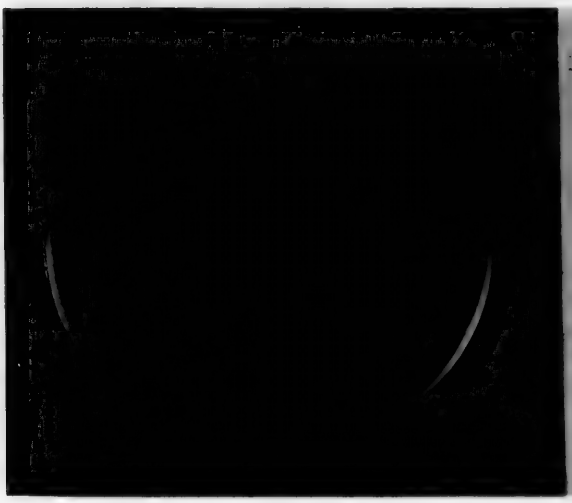

Fig. 3.

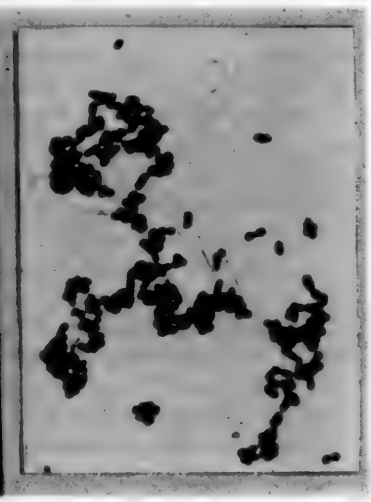

Fig. 4.

Fig. 3.—Small zones of clearing as produced by a streptococcus; ×0.65.
Fig. 4.—Streptococcus from one of the colonies in Fig. 3, stained by Gram's method; ×1100.

To evaluate the possibilities of this medium in a test for caries activity, eighty-seven samples of saliva from forty-four persons aged 18 to 30 years were tested. Control tests were made using tomato agar plate counts, Snyder's bromcresol green dextrose agar, and glucose broth of initial pH 6.2 for the determination of the final pH produced. Results were compared with evidence obtained from clinical examination and bite-wing radiographs. A summary of the results is given in Table I.

TABLE I

CLINICAL SUSCEPTIBILITY	NUMBER OF PATIENTS	NUMBER OF SALIVARY SAMPLES	NUMBER OF SALIVARY SAMPLES CORRELATING WITH CLINICAL FINDINGS			
			PHOSPHATE TEST	TOMATO AGAR TEST	SNYDER TEST	FINAL pH TEST
High	16	37	25	17	23	21
Moderate	24	41	30	22	21	17
Low or immune	4	9	4	6	6	–
Total	44	87	59	45	50	38

DISCUSSION

In the small group studied the results of the tests show fair correlation with the clinical data, but experiments on large numbers of persons with varying degrees of caries susceptibility are needed to determine the usefulness of the new procedure. It may be possible to improve the procedure on further study. It is hoped to make it sufficiently simple to serve as a practical test, for which it is particularly well suited, since the colonies are readily distinguishable by naked-eye observation.

The diabasic calcium phosphate, which is added from a water suspension, prevents diffusion through the medium of the acid formed by the acidogenic organisms. If calcium carbonate is used, as advocated by Wade, Smiley, and Boruff,[24] the acid from strongly acidogenic colonies clears the entire plate making it impossible to distinguish the acid-forming from the non-acid-forming colonies. A separate experiment showed that clearing of the phosphate takes place at pH 4.0. It was found that the color of the indicator changes from purple to yellow between the pH range of 5.35 to 4.5.

SUMMARY

A preliminary report is presented on a new medium for the estimation of acidogenic bacteria in saliva. It appears to have certain advantages over media previously used for the purpose because it supports the growth of many different types of bacteria but permits the quantitative differentiation of strongly acidogenic organisms from those which are weakly acidogenic, non-acidogenic, or alkali-producing. The differentiation is clear cut, and the colonies may be recognized by naked-eye examination.

As a test for caries susceptibility, this procedure, in common with all in vitro tests, shows only the relative numbers of organisms capable of producing acid of a sufficiently low pH to decalcify enamel in the presence of a suitable substrate. If the preliminary work here reported is supported by further clinical and laboratory investigation, and the results of the tests can be correlated with the factors responsible for the rate of neutralization in the mouth of the

patient concerned, it appears that this medium may have a place as an adjunct to diagnosis preparatory to and during the institution of a program of preventive dentistry. Since the oral flora is known to fluctuate with the rise and fall of the intake of carbohydrates, a series of tests at regular intervals will be found to be of more significance than a single test.

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THE INFLUENCE OF AGE IN THE DEVELOPMENT OF DENTAL CARIES IN THE RAT (RATTUS NORVEGICUS)

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INTRODUCTION

THE primary object of the present investigation was the establishment of the relationship between the age of experimental animals and their susceptibility to dental caries. Incidental to the pursuit of this goal, observations were made on the caries-retarding effect of rice flour in the diet.

During the past decade, a number of investigators have concluded that a relationship exists between age and susceptibility to caries in human beings (Hill,[1] Moore,[2] Burrill[3]). Hodge[4] observed that rats one year old developed caries more slowly than did weanlings on the same diet. In addition he summarized the age-susceptibility investigations of many workers.

The present experiment was conducted with albino rats from Hunt and Hoppert's genetically caries-susceptible strain. This choice was made because of the rapidity with which these animals develop caries, thereby making them convenient material for an investigation of this nature. Over a period of ten years, Hunt and Hoppert[5-8] have succeeded in breeding two strains of rats, one strain caries-susceptible and the other caries-resistant. Dental caries was induced in both lines by the rats being placed when 35 days old on the following diet: 66 per cent (by weight) coarsely ground polished rice, 30 per cent whole milk powder, 3 per cent alfalfa leaf meal, and 1 per cent sodium chloride. According to Hoppert, Webber, and Canniff[9, 10] certain cereals when coarsely ground are more conducive to caries development than the same cereals when more finely ground. Accordingly, a "fine" rice mixture (obtained by grinding the rice into smaller particles, but otherwise the same as the "coarse" rice mixture) was used when it was not desired to produce caries. The young animals of both strains were supplied with the fine ration until they were thirty-five days old, whereupon they were furnished the coarse ration. The relative proportions of coarse and finer rice particles are indicated in Table I by a typical screening test of the two types of diet.

After the change from the fine to the coarse mixture, the lower molars were examined under a strong light by the unaided eye at fourteen-day intervals. "Caries time" is the number of days elapsing between the diet change and the date dental caries is first seen. It is an index of caries susceptibility.

Types of cages, source of drinking water, laboratory room temperature, and other environmental factors have been described in an earlier report by Hunt, Hoppert, and Erwin.[8]

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These investigators proved that with environmental factors held fairly constant, most of the difference between the susceptible and resistant lines was hereditary. It is not known whether the genes for caries susceptibility act through producing an effect on the structure of the teeth, on the composition of saliva, on the arrangement of the teeth in the dental arch, or on any of the other factors suggested by the various phases of research into the etiology of dental caries. But it is known that the difference between the strains is far too great to be reasonably explained as a chance occurrence.

TABLE I

COMPARISON OF DEGREES OF DIVISION OF RICE PARTICLES IN THE FINE RICE AND COARSE RICE RATIONS

	FINE RICE	COARSE RICE
Retained on 20-mesh screen	1%	71%
Retained on 40-mesh screen	56%	19%
Retained on 60-mesh screen	21%	
Retained on 80-mesh screen	8%	10%
Retained on 100-mesh screen	4%	
Passed through 100-mesh screen	10%	
	100%	100%

These data were obtained from a typical screening test during the experiment.

Fifteen animals from Hunt and Hoppert's eighth generation of the susceptible strain, which had already developed caries, were chosen as breeders in this experiment. According to Hunt, Hoppert, and Erwin's[8] earlier studies, these breeders and their offspring were probably homozygous for caries susceptibility (that is, they had all the genes for early caries development which are to be found in their strain). The offspring were the experimental animals of this study. Later more experimental rats were needed. They were obtained by the mating of some of the earlier animals which had already developed caries and had thus terminated their usefulness for further observations. Hunt and Hoppert's ninth generation was the control group for the present investigation.

Radusch[11] reported important variation in the diagnosis of dental caries by different examiners observing the same human teeth. To make this personal error constant, one observer (C. A. H.) made almost all of the examinations of both the control and the experimental rats. In a few instances where this was impractical, the examinations were made by only one other person (H. R. H.).

To determine the effect of age, it was originally planned that some of the experimental rats were to be left on the fine rice mixture to the age of 100 days to prevent the appearance of caries. They were then to be fed the coarse ration until caries was first seen. A second group was to be left on the fine mixture until 150 days old and then transferred to the coarse diet. The mean caries times of the two groups were then to have been determined separately, beginning at 100 or 150 days of age rather than at 35 days, as was done with the control group. Then a comparison of the caries times of the control group, the 100-day group, and the 150-day group should show the effect of age on caries development, if such an effect existed.

This plan failed because the first eleven animals on the fine feed developed caries before the age of 100 days, when they were to have been placed on the

coarse rice diet. A revision of plan was mandatory. A third variation of the ration was devised based on the proposition that if the fine rice mixture were less conducive to the development of caries than the coarse, perhaps a still finer rice would delay caries development even longer. Therefore, commercial rice flour was substituted for ground rice in making up the mixture. Otherwise, the diet remained the same. This commercial rice flour was produced from polished rice, and did not contain substances added for enrichment. This flour had been passed through silk bolting cloth to insure extreme fineness.

One group of animals was maintained on the "rice flour" mixture until 100 days old, another until 150 days old. At the stated ages, each group was placed on the coarse ration. No changes were made in the management of the controls, these animals being left on the fine ration until 35 days old, at which time they were given the coarse mixture.

DATA AND DISCUSSION

The data thus obtained are summarized in Table II. For each rat the caries time was the age in days at the date when tooth decay was observed, minus the age in days at which the animal was placed on the coarse rice mixture. Throughout this report, the standard errors of means, standard deviations and differences between means are used rather than probable errors of these values.* The standard deviations and means were calculated in the usual manner.

The 100-day group, as described in Table II, requires no discussion at this point. In the 150-day group, it will be noted that the standard deviation was surprisingly large. This is partially due to the fact that two animals showed phenomenal resistance to the carious process, having caries times of 223 days and 283 days, respectively, while the mean caries time of their group was only 52.5 days. They alone raise the mean of their group by 9.7 days. Dr. W. D. Baten, formerly Professor of Mathematics at Michigan State College, advised eliminating these two animals from the data. Mathematical comparison of the groups is misleading if one permits only two animals to increase the mean and standard deviation of their group to an exorbitant extent. The cause for the outstanding resistance of these two animals is not definitely known. Since this experiment was performed, we have found (H. R. H. and C. A. H.—data as yet unpublished) that sometimes the upper molars are damaged or destroyed by fracturing. The then unopposed lower molars (the lower molars are the only teeth examined routinely in our experiments) show more caries resistance than otherwise. It is possible that such may have been the case with these two rats. The data pertaining to the 150-day group are adjusted in Table II in the section marked "150-day group—corrected," by omission of these two animals.

The "fine rice ration group" of Table II is composed of twenty-six rats, eleven of which developed caries in unmeasured time, as previously stated. The remaining fifteen animals were left on the fine diet and showed dental caries at a mean age of 87.3 ± 7.3 days. Although there was no diet change after weaning, 35 days must nevertheless be subtracted from their mean age at caries develop-

*The standard error equals the probable error divided by 0.6745.

ment to obtain a caries time comparable to that of the control group. A moment's reflection will show that this is permissible, because to the age of 35 days both the control and this group were fed the fine rice mixture. The difference between them is simply that at the age of 35 days, the control group was changed to the coarse rice diet, while the other group was left on the fine ration.

TABLE II

SUMMARY OF DATA FOR ALL EXPERIMENTAL GROUPS AND THE CONTROL

			NUMBER OF INDIVIDUALS
100-day group (developed caries on coarse diet after receiving flour diet to age of 100 days)			49
Mean age when caries was observed	157.6 ± 4.2 days		
Mean caries time	57.6 ± 4.2 days		
Standard deviation	29.6 ± 3.0 days		
Variance	876.16		
150-day group (developed caries on coarse diet after receiving flour diet to age of 150 days)			43
Mean age when caries was observed	202.5 ± 8.5 days		
Mean caries time	52.5 ± 8.5 days		
Standard deviation	55.9 ± 6.0 days		
Variance	3124.81		
150-day group—corrected		(41)	
Mean age when caries was observed	192.8 ± 5.4 days		
Mean caries time	42.8 ± 5.4 days		
Standard deviation	34.3 ± 3.8 days		
Variance	1176.49		
Fine rice ration group (developed caries on fine rice ration without coarse diet)			26
Caries time not measured but less than 100 days		(11)	
Caries time measured		(15)	
Mean age when caries was observed	87.3 ± 7.3 days		
Mean caries time	52.3 ± 7.3 days		
Standard deviation	28.3 ± 5.1 days		
Variance	800.89		
Group developing caries on rice flour ration only			6
Mean age when caries was observed	131.0 days		
Total number of animals participating in experiment, exclusive of control group			124
Control (Hunt and Hoppert's ninth generation of the susceptible strain)			86
Mean age when caries was observed	64.3 ± 1.0 days		
Mean caries time	29.3 ± 1.0 days		
Standard deviation	9.3 ± 0.7 days		
Variance	86.49		
Grand total of animals			210

During the experiment, six animals developed caries on the rice flour diet without attaining sufficient age to be placed in the group for which they were intended. As shown in Table II, the mean age at caries development for these animals was 131 days. Two of these animals were 147 days old when tooth decay was observed, and one was of each of the following ages: 142 days, 139 days, 120 days, and 91 days. These six animals suggest some persisting genetic variability in the susceptible line, unknown and uncontrolled environmental causes, or both.

COMPARISONS OF EXPERIMENTAL GROUPS WITH EACH OTHER AND WITH THE CONTROL GROUP

The comparisons of the experimental groups with each other and with the control are summarized in Table III. In order to determine whether or not the differences between the mean caries times of the various groups are significant, the "t-value" for each difference was calculated.[12]

TABLE III
A COMPARISON OF THE MEANS OF THE VARIOUS GROUPS

GROUPS BEING COMPARED		DIFFERENCE IN DAYS	DEGREES OF FREEDOM	VALUES OF t	SIGNIFICANCE
GREATER MEAN	LESSER MEAN				
100-day group	Control group	28.3 ± 4.3	133	8.161	1%
150-day group corrected	Control group	13.5 ± 5.5	125	3.373	1%
100-day group group	150-day group corrected	14.8 ± 6.8	88	2.171	5%
Fine feed group	Control group	23.0 ± 7.4	99	5.998	1%
100-day group	Fine feed group	5.3 ± 8.4	62	0.610	n.s.
Fine feed group	150-day group corrected	9.5 ± 9.1	54	0.960	n.s.

Significance level is indicated by the corresponding percentage; n.s. means that the difference is not statistically significant. Differences which are significant at the 5 per cent level are large enough, so that considering the number of animals involved (from which the degrees of freedom are calculated), there is less than one chance in twenty that the difference is due to sampling error. Differences significant at the 1 per cent level have less than one chance in 100 of being due to sampling error. The formula by which "t" is calculated is:

$$t = \frac{M_1 - M_2}{\sqrt{\frac{\sum n_1 d^2 + \sum n_2 d^2}{n_1 + n_2 - 2}}} \sqrt{\frac{n_1 \cdot n_2}{n_1 + n_2}}$$

In this formula, M_1 and M_2 are the means of the groups being compared; n equals the number of individuals in a group; d represents deviation from the mean of the group by various classes within the group. Degrees of freedom and values of t must be checked against prepared tables to obtain the significance level.

It will be noted from Table III that placing these rats on the coarse diet at 100 days of age instead of at 35 days delayed tooth decay by approximately 28 days. There is less than one chance in 100 that this difference is due to errors in sampling. However, one animal developed caries when less than 100 days old, while still consuming the rice flour ration. This one animal, while probably highly susceptible, could not possibly reduce the mean caries time of the 100-day group to a point where the difference from the control would no longer be significant.

The "150-day group—corrected" exceeds the control group by 13.5 days. This difference is significant at the 1 per cent level. Likewise, the mean caries time of the 100-day group is about 14.8 days greater than for the corrected 150-day group. This difference is significant at the 5 per cent level.

To sum up these three comparisons, 35-day-old animals are highly susceptible to tooth decay, 100-day-old animals appear to be considerably more resistant, and 150-day-old animals are intermediate. But before such conclusions are drawn some sources of error should be examined.

A precise determination of the effect of age alone on resistance is difficult, because these susceptible rats will sometimes develop tooth cavities when feeding on the rice flour mixture only, if given sufficient time. Possibly all of the susceptible rats of the generation used would have done this if left on the diet long enough. There was probably still some genetic variability among the susceptible animals used in spite of six or seven generations of intensive inbreeding. Perhaps eating the coarse rice mixture is such severe treatment that even a genetically somewhat variable population shows fairly uniform response to this diet, and the real genetic differences are brought out by the milder and more discriminating action of the rice flour diet. At least we know that *some* animals develop cavities on this latter diet. Only one showed cavities before 100 days, so that the caries time of the 100-day group is fairly reliable. More suspicion attaches to the caries time of the 150-day animals because five rats showed dental caries before that age while still eating the rice flour mixture. It is conceivable that the 150-day group was selected somewhat for resistance because the five rats which developed caries while still on the flour mixture could not be included. Therefore the mean caries time of the 150-day animals is possibly too high, though we should not ignore the two rats that were excluded because of their long caries times.

One need not seriously doubt the validity of the control rats because they were in cages with the fine rice mixture. They were in these cages for only 35 days, and the fifteen animals which were confined to the fine ration indefinitely did not, on the average, show caries until 87.3 days old. Thus it is not likely that there was any significant development of caries in the control animals before the age of 35 days.

Table III also shows a difference of approximately 23 days between the means of the fine rice ration group and the control group, which is significant at the 1 per cent level. The differences between the fine feed group on the one hand, and the 100-day and 150-day groups on the other, are not significant. Thus it appears that the delay in the carious process associated with increased age is of approximately the same order as the delay brought about by substituting the fine rice diet for the coarse rice mixture.

A study of Table II shows a considerable amount of variation among the variance values of the different groups, leading one to speculate that the tendency of the caries times of the individual animals within a group to cluster about the mean of the group is not the same in every instance. In fact, mathematically, this can be shown to be true. However, it is deemed better to refrain from making any definite statements about this topic until a larger fund of data is available.

It would be profitable to study the age effect in dental caries more extensively. This report compares only three age groups. Studies on groups of intermediate ages and at ages beyond 150 days would doubtless reveal some interesting relationships. The present research demonstrates primarily that there is

an increase in resistance, and possibly also in variability of resistance to the carious process, concomitant with increasing age in this genetic line. The practical implication is that in using rats as experimental animals in the study of dental caries, one must keep in mind that age is a factor in susceptibility, and make provision for the control of this variable.

SUMMARY

The degree of susceptibility to dental caries in genetically susceptible rats is partially dependent on age. The teeth of our rats are significantly more resistant to decay at 100 days and at 150 days of age than at 35 days. When 100 days old, the susceptible rats have a longer caries time than when 150 days old.

The results of Hoppert, Webber, and Canniff[9, 10] are again confirmed, and several additional facts are discovered. (a) Substitution of the coarse rice for the fine rice in the ration at 35 days of age results in prompt tooth decay in genetically susceptible rats; the difference induced by the diet change is approximately 23 days of caries time. (b) Substitution of rice flour for fine rice in the ration prevented the development of caries in susceptible rats before the ages of 100 or 150 days in 94 per cent of the animals tested. The potency of the environment is clearly indicated by this fact.

Two genetically susceptible animals exhibited unexpected and remarkable resistance to tooth decay when placed on the coarse rice ration at 150 days of age.

Six animals showed extreme susceptibility to dental decay, so that they were unable to attain the age of 150 days on the rice flour ration without developing caries.

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A METHOD FOR THE QUANTITATIVE ESTIMATION OF INTERPROXIMAL CAVITY SIZE

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A RECENT study[1] of caries activity in children has served to emphasize the importance of long-term studies in individual subjects. Such an approach must evaluate the progress of untreated lesions as well as the occurrence of new cavities. The former cannot be estimated quantitatively by any of the more frequently used scoring methods, such as those of Klein, Palmer, and Knutson,[2] Bodecker,[3] Mellanby,[4] Day and Sedwick,[5] and Boyd.[6] The application of a quantitative method not only would introduce objective analysis to this problem but also would satisfy a general need for exact information on the rate of dissolution of tooth substance. Despite the importance of such information to an understanding of the caries process, only one brief report[7] has come to our attention. Chemical methods were employed to measure the loss of enamel salt which was found to be approximately 1 mg. of apatite per month. The need for more data is apparent.

Shepherd[8] described a technic whereby the size of interproximal cavities is expressed in terms of the area recorded upon bite-wing radiographs. This approach is capable of improvement since the volume of a cavity, rather than its cross-sectional area, is more properly descriptive of size. It occurred to us that if some simple relationship existed between the two quantities, volume could be estimated from the observed area. The present work deals with an attempt to define such a relationship by the examination of a series of cavities in extracted teeth.

METHODS

Forty-eight extracted permanent teeth (and one deciduous tooth) containing a total of fifty-five proximal cavities were selected for study. With few exceptions, enamel destruction was limited entirely to the proximal surfaces; where extension to adjacent surfaces did occur, the involvement was minimal. Cavity size never exceeded, and rarely approached, one-quarter the volume of the crown.

Experimental procedure was divided into two parts: (1) determination of cavity area as observed on the dental radiograph, and (2) determination of cavity volume.

Determination of Cavity Area:

The lingual surface of each tooth was attached to Eastman radiatized intraoral dental film by means of soft wax so that the long axis of the crown paralleled the film. X-rays were directed through the buccal surface, perpendicular

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to the plane of the film. The distance from tip of cone to surface of film was two inches. Exposure time was 2½ seconds for anterior and 3¼ seconds for posterior teeth.

After processing, the radiograph was placed in a standard photographic enlarger and projected onto 1-cm. graph paper at somewhat less than × 10 linear magnification. The outline of the carious lesion was traced on the paper. In all cases three successive tracings were made. These together with a number of squares 1 cm. on an edge were cut out and weighed individually on a torsion balance. Average values were calculated. The area of the paper tracing in square centimeters was calculated from the following equation:

$$\text{Area of tracing} = \text{Area of standard (1 sq. cm.)} \times \frac{\text{wt. of tracing}}{\text{wt. of standard}}$$

Values so obtained were multiplied by the appropriately derived factor in order to reduce them to the actual cavity area observed on the radiograph, expressed in square millimeters.

We believe that the cut-out technic for determining projected area represents a real improvement over the direct-count-of-squares originally suggested by Shepherd. Its advantages are speed and greater freedom from systematic error.

Determination of Cavity Volume:

All white, friable enamel and softened dentin were removed with sharp spoon excavators. Care was observed in preserving sound tooth structure. The cavity was completely filled with temporary stopping and shaped to the contour of the tooth. The material was then removed and weighed on an analytical balance.* Cavity volume was calculated from the expression:

$$\text{Volume} = \frac{\text{weight of filling}}{\text{density of temporary stopping}}$$

Since the filling material was subjected to heat and compression in the course of insertion, it seemed possible that its initial density† may have been altered. In order to check on this point, the following experiment was undertaken. Six successive fillings were placed in a large molar cavity. Each was removed in one piece, weighed, and the volume calculated as described. In addition, volume was obtained directly from the weight of water displaced by the filling as determined upon a hydrostatic balance. The results of both procedures were in close agreement (Table I) so that little if any change in density occurred during insertion.

Accuracy and Precision of Methods:

The x-ray technic employed was assumed to provide an accurate measure of cavity dimensions. Likewise, the method of cavity excavation was assumed to remove all decalcified material. While such assumptions are probably valid, they deserve further investigation.

* The weight of a few large fillings was derived by difference between tooth weight before and after filling.

†Density varied somewhat from one stick of material to another but was quite constant for a given sample. Values observed were 2.82, 2.86, 2.86, and 3.22 Gm. per cubic centimeter.

TABLE I
VOLUME OF MOLAR FILLING DETERMINED BY TWO DIFFERENT PROCEDURES

FILLING NUMBER	VOLUME (CU. MM.)	
	DIRECT* METHOD	INDIRECT† METHOD
1	33.	33.3
2	34.	34.2
3	35.	33.9
4	34.	34.2
5	33.	34.2
6	32.	33.3
Average	34.	33.9

*The term "direct" indicates that volume was derived from the weight of water displaced by filling as determined upon a hydrostatic balance.

†"Indirect" indicates that volume was calculated from the weight of filling and the known density (prior to insertion) of the filling material.

The precision of area measurement was examined in a series of successive tracings made from the radiographs of four cavities. Precision of volume determination was examined by the repeated filling of six cavities. The degree of reproducibility of both methods is shown in Table II. Variation was such that results were expressed to no more than two significant figures and were always limited to the first decimal place. As might have been anticipated, both methods were less precise when applied to larger cavities.

It should be emphasized that the reproducibility of the x-ray technic itself was not investigated. However, several exposures at an anode-film distance

TABLE II
REPRODUCIBILITY OF AREA AND VOLUME MEASUREMENTS

TOOTH	NUMBER OF OBSERVATIONS	CAVITY AREA (SQ. MM.) MEAN ± STANDARD DEVIATION*
	Area	
Incisor	16	5.10 ± 0.30
Canine	20	4.01 ± 0.12
Premolar	20	4.85 ± 0.23
Molar	20	9.97 ± 0.52
TOOTH	NUMBER OF OBSERVATIONS†	CAVITY VOLUME (CU. MM.) MEAN ± STANDARD DEVIATION*
	Volume	
Premolar	1	1.4
	6	1.3 ± 0.1
Premolar	1	2.6
	6	2.7 ± 0.2
Molar	1	3.0
	6	3.0 ± 0.1
Molar	1	9.10
	6	9.33 ± 0.26
Premolar	1	13.2
	6	14.3 ± 0.2
	3	13.4 ± 0.2
	3	14.5 ± 0.1
Molar	1	31.0
	6	33.9 ± 0.4

*Where N < 20 the unbiased estimate of the standard deviation (σ) is given.

$$\sigma = \sqrt{\frac{\Sigma\ d^2}{N\text{-}1}}$$

†Only consecutive observations were used in the calculation of each mean. Single observations and individual groups of observations reported for the same cavity were separated from each other by long intervals of time.

TABLE III
AREA-VOLUME DATA FOR THE FIFTY-FIVE CAVITIES EXAMINED

AREA (SQ. MM.)	VOLUME (CU. MM.)	AREA (SQ. MM.)	VOLUME (CU. MM.)
Incisor		*Canine*	
.2	.7	.9	.7
.8	1.5	1.6	4.4
1.5	1.4	4.0	9.4
1.5	1.6	5.1	13.
1.6	2.2	5.1	13.
3.1	3.9	7.4	22.
3.4	4.3	*Premolar*	
3.6	7.7	.3	.8
4.1	5.1	1.0	.9
4.2	6.1	1.0	2.1
4.8	8.8	1.0	1.0
5.1	16.	1.0	2.6
7.2	17.	1.7	3.4
9.9	29.	1.9	1.3
13.	30.	2.0	2.7
		2.0	3.4
Molar		2.4	7.9
.4	1.0	3.2	7.9
.7	3.0	3.2	11.
1.9	6.0	4.2	16.
2.3	5.1	4.9	13.
2.4*	3.2	4.9	14.
3.0	7.3	5.2	6.5
4.2	14.	5.4	15.
5.1	9.1	5.4	16.
8.6	37.	5.8	13.
10.	33.	8.0	15.
13.	57.	8.1	19.
14.	42.	8.1	26.

*Deciduous tooth.

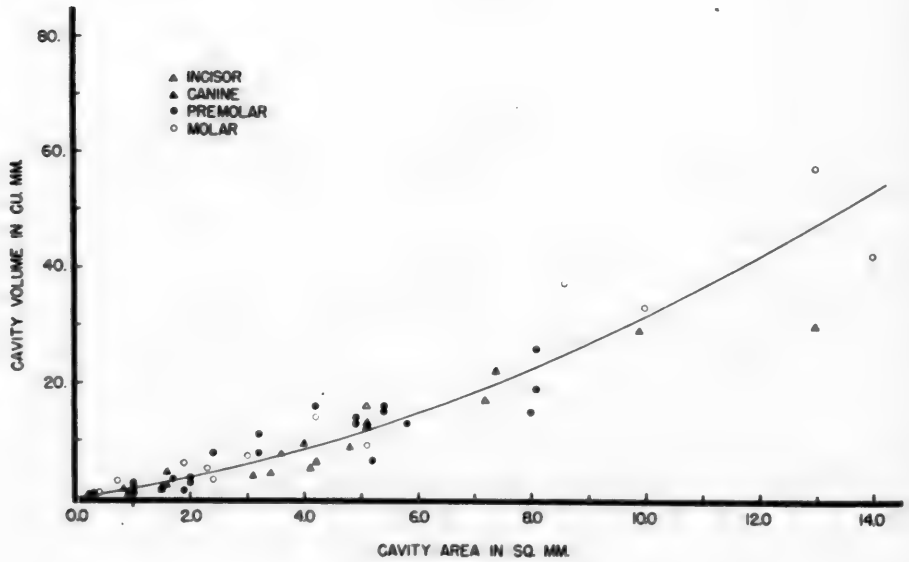

Fig. 1.

of 22 inches produced better definition of cavity outline than did the short distance employed throughout the study. These results together with the reports of Fitzgerald[9, 10] indicate that the long anode-to-film distance may be of advantage both in extracted teeth and in the mouth.

RESULTS

The experimental data for each of the fifty-five cavities are listed in Table III. These values were plotted and a curve was drawn by inspection (Fig. 1). The expression, $V = A,^{1.5}$ where V = volume and A = area, appeared to satisfy the requirements of the curve. Cavities in different morphologic types of teeth in some cases tended to deviate from the curve; e.g., incisor cavities fell below while molar cavities were somewhat above. Because of the relatively small number of observations, the entire body of data was considered as a whole. It is possible that further information may result in slightly different curves characteristic of the type of tooth.

DISCUSSION

Application of our findings to clinical problems would proceed along the following lines: Bite-wing radiographs of suitable quality are taken. The film is projected in a photographic enlarger and the area of each cavity is determined by the cut-out technic. Volume is derived by calculation from the equation, $V = A,^{1.5}$ or by reference to the graph. Two or more determinations separated by suitable periods of time define the rate of cavity increase.

The quantitative relation between cavity area and volume which has been suggested is offered only in a preliminary way. A number of sources of error must be investigated more closely before the method can be applied to problems of clinical caries with complete confidence. The chief question to be clarified is the reliability of bite-wing x-ray technic. Nevertheless, there is every reason to believe that a perfectly valid and reliable method can be developed. That a procedure of the sort we have been developing can have clinical usefulness is shown by the application of the identical principle in another field. Annegers and Ivy[11] report the estimation of changes in volume of gastric contents from changes in area of the projected x-ray shadow of the stomach.

SUMMARY

A method has been described for relating the volume of an interproximal cavity to its area as measured on bite-wing radiographs. The equation, $V = A,^{1.5}$ where V = volume and A = area, was descriptive of this relation in fifty-five cavities. The method may have some application in the study of the rate of tooth dissolution and in the clinical evaluation of dental caries.

The authors wish to express their appreciation to Dr. Basil G. Bibby who read the original manuscript and suggested a number of important changes.

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A NOTE ON THE ESTIMATION OF PHOSPHATE IN SALIVA

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In the course of an investigation on the chemical mechanisms involved in dental caries it has been necessary to analyze salivas for inorganic and organic phosphate. Since the results obtained differ from those published by Eggers Lura,[1] we feel that a few comments on the methods used would aid in the solution of this fundamental problem.

In Lura's paper there is, we believe, a serious misprint on page 206 in the paragraph headed, "Calculation of Organic Fractions," where the plus signs should be changed to minus signs throughout. We also noted in passing that in the list of references, the paper ascribed to Glock, Murray, and Pincus: *Biochem. J.* **28**: 720, 1934, should be listed, we believe, as vol. 32, page 2096, 1938, and that the Giri reference preceding this has the wrong page number, 386 instead of 306.

In our research we found that the total acid-soluble phosphate was the same as the total phosphate within experimental error. This means that there is no acid insoluble phosphate fraction either organic or inorganic. Lura's available results bear out this contention, although he has published tables containing columns headed, Total Phosphate, Total Acid Soluble Phosphate, as if these were different (Tables VI and VII). In Table VIII he arbitrarily assumes that the differences between total phosphate and total acid soluble phosphate is organic phosphate, even though the said results, according to his statement at the top of page 209, "were so insignificant that they did not surpass the standard error."

In other words, we are of the opinion that Tables VI and VII are attempting to portray the same information; namely, the amount of organic phosphate in saliva.

The results Lura presents are open to the objection that he has taken no precaution to prevent the hydrolysis of labile phosphate esters. In our experiments the saliva on occasion stood twenty-four hours in a refrigerator before analysis, and such hydrolysis, we found, could be prevented by the addition of 50 ppm of sodium fluoride.

We encountered another rather serious problem in that it was difficult to obtain trichloro-acetic acid filtrates which would remain perfectly clear on the addition of the ammonium molybdate reagent used in our procedure.[2] To overcome this difficulty, it was found necessary to determine the total phosphate in the saliva, precipitate the inorganic phosphate by the addition of magnesia mixture, and then do a total phosphate on the filtrate. The difference between the total phosphate before and after this procedure is a measure of the organic phosphate. Smith[3] encountered a similar difficulty and overcame it by the expedient of precipitating the inorganic phosphate and redissolving it prior to its estimation.

EXPERIMENTAL PROCEDURE—TOTAL PHOSPHATE

One milliliter of saliva was digested with 1.2 ml. of 60 per cent perchloric acid. To this solution was then added 1 ml. of 5 per cent ammonium molybdate and 1 ml. of 1-amino-2-naphthol-4-sulphonic acid reagent. After twenty minutes the blue color was compared with a standard phosphate solution.

ORGANIC PHOSPHATE

To 10 ml. of saliva was added 0.3 ml. of magnesia mixture (calculated excess) without the ammonium hydroxide, and then 0.3 ml. of ammonium hydroxide. After being shaken and after standing for ten minutes, the suspension was filtered and 5 ml. of the filtrate was digested, and the phosphate was determined in the same manner as described for total phosphate. The difference between the total phosphate and the organic phosphate is a measure of the inorganic phosphate. Experiments were performed to demonstrate that the amount of fluoride added did not interfere with the phosphate analysis and that organic phosphates such as glycerophosphates and phenyl phosphates were not occluded by the magnesium ammonium phosphate. The results are tabulated in Table I as follows:

TABLE I
TOTAL, ORGANIC, AND INORGANIC PHOSPHATE IN SALIVA

CASE	T.P. MG. %	O.P. MG. %	I.P. MG. % (BY DIFFERENCE)	O.P./T.P.	LACTOBACILLUS COUNT
1	12.7	1.60	11.1	0.13	0
2	16.7	1.36	15.3	0.08	0
3	10.8	0.77	10.0	0.07	0
4	15.5	0.70	14.8	0.05	0
5	14.0	0.99	13.0	0.07	0
6	13.9	0.59	13.3	0.04	0
7	10.4	0.68	9.7	0.07	100
8	9.3	1.54	7.8	0.15	250
9	9.8	0.98	8.9	0.10	300
10	14.0	1.13	12.9	0.08	450
11	9.8	1.10	8.7	0.12	900
12	12.2	0.77	11.4	0.06	1,000
13	15.0	1.75	13.2	0.12	2,000
14	12.1	0.58	11.5	0.05	2,000
15	11.6	0.93	10.7	0.08	3,000
16	10.4	0.85	9.6	0.09	9,000
17	12.0	0.88	11.1	0.07	10,000
18	16.9	1.08	15.8	0.06	12,000
19	11.5	0.64	10.9	0.06	12,000
20	16.7	0.73	16.0	0.04	12,000
21	9.8	1.36	8.4	0.14	15,000
22	13.7	0.60	13.1	0.04	15,000
23	17.0	0.83	16.2	0.05	30,000
24	13.7	0.95	12.7	0.07	36,000
25	10.9	0.74	10.2	0.07	36,000
26	11.2	0.80	10.4	0.07	45,000
27	10.1	0.59	9.5	0.06	60,000
28	11.7	0.55	11.1	0.05	60,000
29	11.6	0.52	11.1	0.04	75,000
30	17.2	0.92	16.3	0.05	75,000
31	18.7	1.30	17.4	0.07	120,000
32	11.5	0.73	10.2	0.06	120,000
33	14.5	0.40	14.1	0.03	120,000
34	13.8	1.21	12.6	0.09	300,000
35	11.2	0.94	10.3	0.08	360,000

CONCLUSIONS

The results presented are more uniform than those presented by Eggers Lura. The organic phosphate fraction is much smaller than he found it to be.

The ranges and average values are:

T.P.	from 9.3 to 18.7	Avg. 12.9 mg. per cent
O.P.	from 0.40 to 1.75	Avg. 1.15 mg. per cent
%O.P.	from 3 to 15	Avg. 7.3

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THE ESTIMATION OF PHOSPHATE IN SALIVA
(A Reply)

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The comment by Dr. Muriel Davies and Dr. James Rae has been presented to me, and I should like to add some few remarks.

I sincerely regret the misprint on page 206, but it was due to the difficulties in getting a second proof forwarded to Denmark in time. I am happy to have it corrected.

The fact that your results are more uniform than those presented by me and that the organic fraction found by you is much smaller than that found by me, may be explained by the following observations:

First: Your storage of saliva twenty-four hours before analysis. Even by refrigeration and by addition of sodium fluoride the values can change considerably in this space of time.

Second: Your technic is quite different from mine. Unfortunately, at present, we have no completely satisfying procedure for determining and identifying the phosphate esters, especially the labile phosphate esters.

In my technic the quantity of inorganic phosphate is determined by direct colorimetry of the solution. You are quite right that this technic is open to the objection that not only the real inorganic phosphate is included in the results, but also some inorganic phosphate split off from the acid-labile phosphate esters (especially guanidine phosphate) during the sixty minutes' comparison time. It is possible that some of the higher inorganic values in my experiments may be explained in this manner. On the other hand, my determination of total phosphate by using the "wet-ashing-procedure" seems to be more perfect than the digestion by perchloric acid used in your experiment. You have to remember that some of the phosphate liberated by the digestion will be converted into inorganic pyrophosphate, a phosphoric compound which gives no color in the colorimetry. In my procedure, this error is avoided by addition of Perhydrol and water, so that my total P values will be much higher than yours.

In your determination of inorganic phosphate, you are making a precipitation by alkaline reaction, but you forget that some alkaline-labile phosphate esters may be hydrolyzed during this procedure. I think a better method would be that indicated by Lipmann in one of his investigations on acetyl phosphoric acid. In this work the inorganic P is precipitated quantitatively by a reaction between pH 5 and pH 7 by means of $CaCl_2$ dissolved in 30 per cent ethyl alcohol. As a matter of fact, your precipitation of the inorganic phosphate is not at all quantitative. This contention may be checked in the following manner:

To a solution containing some known quantity of inorganic phosphate a little radioactive phosphate (some few gamma of P^{32}) dissolved in water is added. Afterward the precipitation is made in the usual manner with ammonium-magnesium-hydroxide. After removal of the precipitate it appears that the supernatant liquid which ought to be free from phosphate still contains a considerable amount of unprecipitated radioactive phosphate (10-15 per cent).

In short, my conclusions are the following:

1. I have used a technic which gives greater values of inorganic phosphate, and especially of total phosphate.

2. You have used a technic which gives smaller values, especially of total P.

3. Both my procedure and yours are open to considerable experimental errors, but for the present, unfortunately, we have no better technic. It is difficult to decide which of the methods is the better one.

The aim of my paper was only to point out a single experimental error in the investigation of the salivary phosphates, namely, the salivary phosphatases. I know that there are still many other errors and unsolved problems in this area, but I hope that we may succeed in discovering as many of these errors as possible. I shall always be grateful for any objective and rational criticism, and especially for any better proposal.

A DENTAL EXAMINATION RECORD FORM FOR STATISTICAL MACHINE TABULATION

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IT IS now fully a decade since the Hagerstown Dental Examination Form was developed.[1] The statistical processing methods applied to findings recorded on that form have proved of much utility in analysis of epidemiological data in the dental caries problem.[2-4] The Hagerstown Dental Recording System requires that the dentist-examiner learn a code corresponding with his direct observations on the teeth and mouth. The appropriate symbols making up the code are called out by the dentist-examiner to a clerical assistant who writes the code symbols directly on the examination form. In the subsequent transfer of the dental findings to Hollerith punch cards, the code symbols which appear on the examination form are punched directly onto the card. Although this procedure eliminates a good amount of the usual clerical manipulation of dental examination forms, hand punching for all the data is required.

In the decade which has elapsed since the Hagerstown Form was first introduced, several improvements and adaptations of the basic form have been made in connection with a variety of special studies. All of these changes have been in the direction of simplification in order that the dentist-examiner might give maximum attention to the dental examination per se, and in order to reduce, to the greatest extent, subsequent clerical handling of the record form.

The development of mark sensing,† makes it possible to introduce further simplifications. Using an appropriately designed form, the observer calls out his findings to a clerk who marks them with an electrographic pencil in precoded spaces. The cards so marked are then ready for mechanical punching. This procedure eliminates entirely the usual intermediate steps of coding, hand punching, and hand verification for a majority of the items to be tabulated.

Design of a mark sense record form for dental examination findings was directly stimulated by the experience of one of the authors (M. K.) in the development of such cards for tuberculosis clinic, mass x-ray, and sanatorium records. It is the purpose of the present communication to describe a particular mark sense card which has been designed specifically for dental examinations. The card has been tested in the field‡ and found to be exceptionally suitable for recording the dental status of population groups.

Description of Examination Record Form:

The examination record form consists of a manila card measuring 7⅜ in.

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**International Office of Health Relations.

†The mark sense principle takes advantage of the fact that when cards, marked with a special mark sense pencil containing lead with a high graphite content, are fed through an IBM Mark Sense Reproducer, electric current flows through the graphite marks and causes holes to be punched in certain preselected columns.

‡The form has been tested in the examination of a population group residing at Isle Madame, Cape Breton Island, Nova Scotia; in a cooperative study with Dr. L. Richter of Dalhousie University, Halifax, Nova Scotia.

by 3¼ in., the standard International Business Machine punch card size. The front of the card (Fig. 1) is divided into four major segments. The first segment on the left-hand side of the card provides space for recording such items as the name and address of the person examined, age and sex, marital status, and number of children in family. Space is also provided for a roster of names of persons in the household as well as case and family number. On the bottom of the first segment, space is available for recording summations of findings on the individual teeth as recorded on the mark sense segments of the card. The second segment to the right provides space for checking age and sex. The remaining segments on the front of the card provide space for marking the condition of each tooth space (deciduous or permanent) on the right and left sides of the upper jaw.

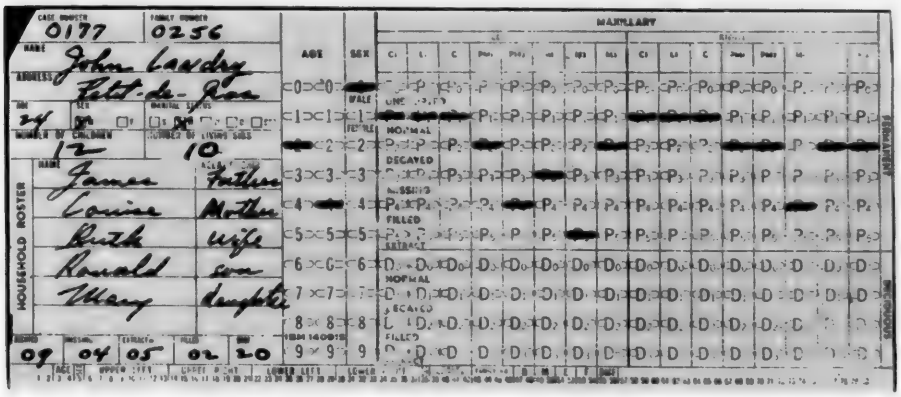

Fig. 1.—Front of mark sense dental examination form showing items recorded by clerk from information called out verbally by dentist-examiner.

The back of the card (Fig. 2) consists of a segment on the left (remarks) for recording special observations and notes relevant to the case. Next, a space is provided for checking observations on occlusion. The remaining segments (except for that on the extreme right of the card where recommendations for dentures are recorded) provide space for recording findings on the individual teeth (deciduous or permanent) on the left and right sides of the lower jaw.

Procedure for Use of the Form:

An electrographic pencil is used to record the information. The clerk interviews the patient and records appropriate items such as: case number, family number, patient's name, age, sex, marital status, number of children and siblings and family roster.* Age and sex are marked in the appropriate mark sense fields. The dentist-examiner then begins his examination and calls out his findings which the clerk marks in the appropriate columns. The observations are made first on the teeth in the left upper jaw beginning with the central

*The information on family was obtained for the Nova Scotia group for analysis of the familial factors in the epidemiology of dental disease. However, the space on the card used here for family information could be utilized to record any other type of information desired instead.

incisor (Fig. 1). When the examination of the upper jaw is completed, the clerk reverses the card where findings on the teeth of the lower jaw are similarly recorded (Fig. 2).

For each tooth space one of the following findings may be marked:

Permanent Teeth		*Deciduous Teeth*	
Unerupted	P0	Normal	D0
Normal	P1	Decayed	D1
Decayed	P2	Filled	D2
Missing	P3	Extracted	D3
Filled	P4		
Extracted	P5		

At the close of the examination period, the clerk adds the number of teeth decayed, the number missing, the number filled, and the number recommended for extraction. These summary figures are entered in the bottom line of the first field on the front of the card and are added together to give the total number of the teeth decayed, missing, or filled (DMF).

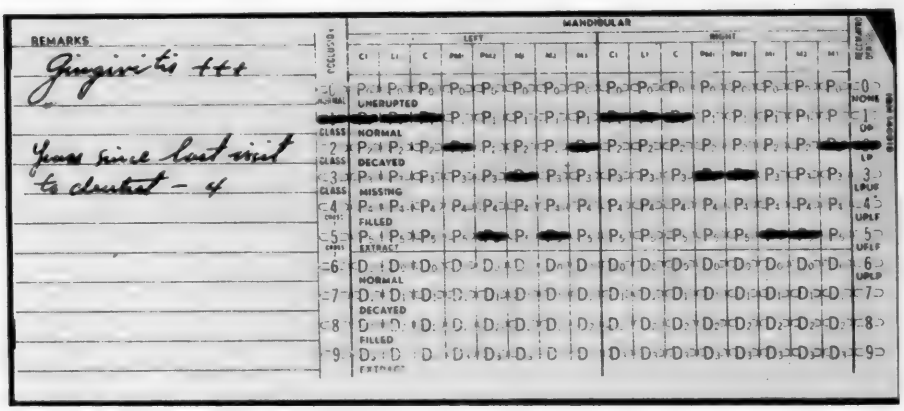

Fig. 2.—Back of mark sense dental examination form showing items recorded by clerk from information called out verbally by dentist-examiner.

The card is punched in two operations (Fig. 3): (1) *Mark sense punching:* All marked items on the front and back of the card activate mechanical punching by the mark sense reproducer into columns 3-38. (2) *Hand punching:* Case number, family number, number of teeth decayed, number missing, number to be extracted, number filled, and total number (DMF) are hand punched into columns 39-56. Additional items, such as the number of deciduous teeth present in the mouth* and the number of such teeth decayed and filled, as well as other observations can be hand punched in the remaining twenty-four columns on the card.

*The original card design does not provide blocked spaces for summarizing findings on the deciduous teeth. However, this is readily accomplished by simply placing the necessary spaces in the summary section of the card.

Utility of the Form:

Ideally, a dental examination should provide a dentist-examiner with facilities which free him completely from distractions so that he can concentrate his entire attention on the oral examination during which he can call out his findings verbally for recording. In many instances in current practice, the dentist-examiner records the findings himself, thus interrupting his observations and therefore interfering with the examination. In other instances, the findings may be dictated into a voice-recording machine and later transcribed by a clerk. Following these manipulations, the record form is coded, hand punched, and verified. Only after considerable handling are the records ready for tabulation.

Use of the form described in the present communication eliminates most of the aforementioned steps. Clerical handling is reduced to a minimum. Many errors which frequently occur through hand processing of statistical record forms are avoided. The most attractive feature of the use of the mark sense form is the fact that the dentist-examiner can give maximum attention to the mouth examination per se. An important feature of the form is the fact that

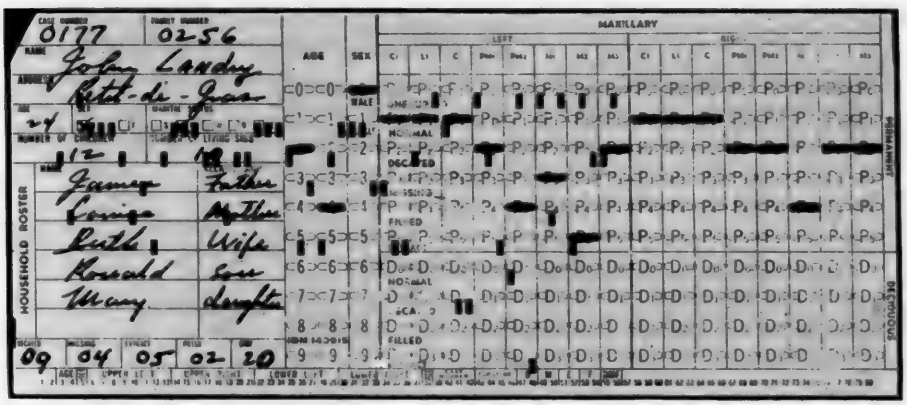

Fig. 3.—The same mark sense dental examination form as shown in Figs. 1 and 2, illustrating appearance of card after items recorded on front and back (see Figs. 1 and 2) have been mechanically punched into columns 3 through 38 and hand punched into columns 39 through 56.

the mark sense cards are readily duplicated and therefore the original schedule cards can be used as file cards and the duplicated set for machine tabulation. A further advantage accrues because summarization of the findings on the teeth is accomplished by adding marks along horizontal rows. For example, the total number of decayed permanent teeth is obtained by adding together all the marked P2 positions on the front and back of the card.

For those situations where a mark sense reproducer is not available, the marked record still remains an efficient self-coded form which facilitates hand punching. For those who may not wish to use a printed card of punch card size, the self-coded features described in this paper could be used on a mimeographed schedule form. Finally, the type of form which has been described lends itself to a variety of applications. Although its use in dental examination has been described, adaptations of the form can be readily developed for clinic

operations where dental services dispensed could be recorded in an efficient manner and from which job analysis of clinic operations and practice could be conveniently accomplished.

Although the card described has the attributes mentioned, it is important to indicate that the mark sense procedure requires extreme care in the physical handling of the cards. The cards must be fed through complicated and delicately balanced electrically activated machines, and serious losses in time and efficiency follow disregard of the special attention required in the marking and handling of the cards. To some extent these precautions may be considered to detract from the utility of the form, especially for those inexperienced in its use. However, the rules for the care and handling are simple and straightforward and no more demanding than other items of attention that must be observed in any careful processing of accurately determined scientific observations.

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WEATHER CONDITIONS AFFECTING PATIENTS' REACTIONS TO LOCAL ANESTHETICS

A Report of 1,319 Consecutive Cases

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INTRODUCTION

HAVING used local anesthetics for over twenty years in operations on the teeth, I have observed numerous instances in which it has been impossible to obtain complete anesthesia of the dental pulp. In certain cases a second injection has produced the desired result, but in some even a third injection has not resulted in pulp anesthesia. A few cases have evidenced minor toxic reactions. While there has been an extensive literature published on the effect of weather change on patients subjected to drugs,[1, 2, 11] a correlation between weather change and degenerative diseases, such as heart disease and diabetes,[3, 4, 11, 12] weather effects upon allergic manifestations,[5, 6] and weather influences upon infectious diseases,[7-10] to my knowledge there has not been any work done on the possible weather-local anesthetic reaction relationship. Some years ago it seemed to me that these occasional abnormal anesthetic reactions might have a seasonal relationship, the impression being that they occurred chiefly in the hot, dry months of August, September, and October. This investigation was begun to determine the possible relationship between weather conditions and patients' reactions to local anesthetics. Only the preliminary phases of this subject are reported.

MATERIALS AND METHODS

All observations are from my practice of general dentistry, exclusive of surgery and orthodontia, in Beverly Hills, California. They cover the period of twenty-four months, from July 1, 1945, to June 30, 1947. In this period there were 4,112 office appointments, during which 1,319 local anesthetics were administered for purposes of cavity preparation upon vital teeth. Multiple cavity preparations were done under many of these anesthetics. Obviously a single patient may be represented several times in the series.

Only three types of injection were used:

1. Mandibular block for the inferior alveolar nerve: used for all operations on the mandibular teeth.
2. Tuberosity block of the posterior division of the superior alveolar nerve: used for all operations on the posterior maxillary teeth, and occasionally adequate for the whole quadrant.
3. Subperiosteal: used for operations on the anterior maxillary teeth, and occasionally on the premolars. This injection is made very slowly to avoid trauma and possible ballooning of the tissues.

No premedication was used at any time. All injections were made by me; my technic and rate of injection have become standardized through experience

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over many years. The same formula of solution* was used in all cases. The age range of patients anesthetized was 5½ to 76 years. There were 413 male patients (31 per cent), and 906 female patients (69 per cent).

An anesthetic reaction has been designated *toxic* when the operator has observed any objective symptom such as blanching, tremor, or tachycardia, or when the patient has reported a subjective symptom such as slight nausea, weakness in the knees or elbows, or a feeling of fullness in the throat. In no instance did any reaction progress to the point of fainting, and the duration of such symptoms has in no instance exceeded three and one-half minutes. These reactions are not to be confused with those of psychic origin. In my office there is no discussion of the use of local anesthesia; the syringe is never seen by the patient and frequently symptoms of numbness are the first knowledge the patient has that an anesthetic has been administered. Typical of psychic reactions are those which were sometimes observed prior to 1944, when surgery was still being done in the office. Some patients who had been anesthetized numerous times for cavity preparation with no reaction whatsoever, became faint, and even at times, nauseated from the same injection when made for purposes of extraction. The large majority of the patients in my practice have been so conditioned for many years to local anesthesia for cavity preparation that more apprehension would be expected from a discussion of the possibility of omitting it than from the anticipation of an injection.

An anesthetic has been designed *abnormal* when there was incomplete anesthesia of the dental pulp, or when the duration of anesthesia was unusually short.

Many of the patients are represented in both categories (i.e., toxic or abnormal), but not at the same time. With one exception there has not been a toxic manifestation with an incomplete anesthetic.

The reactions to anesthetics have been placed day by day upon a graph, the normal appearing above the line established, the abnormal below the line with the sex indicated. The toxic reactions are indicated by a T in a sex symbol on the date of occurrence. A diary of the daily anesthetics has been kept concurrently. At the end of each month a weather summary, supplied by the Weather Bureau of the United States Department of Commerce at Los Angeles, has been used to mark on the graph the weather changes shown.

An attempt by me some years previously to coordinate anesthetic reactions with barometric change was inconclusive. While there is very slight variation in barometric pressures in this area, it was felt that with all other pertinent weather data included and correlated, some relationship to barometric pressures might become evident.

EXPLANATION OF MONTHLY GRAPHS

The daily prevailing wind† is shown at the top by the letter indicating its source.

*NPC, procaine 2 per cent, pontocaine 0.15 per cent, cobefrin 1:10,000, in carpules.

†This was suspected as of possible importance. The prevailing winds are westerly from the Pacific, but winds blowing from the desert, that is, from the east, or locally from the north, cause a sharp decrease in the relative humidity. This results in retention of body static charges, with an observed increased irritability in both human beings and domestic animals. Such a wind blowing with a velocity of 22 miles or more per hour is known locally as a Santa Ana, indicated by SA on the graph.

The daily maximum temperature is recorded next, and below that the daily minimum, the shaded portion between the two curves representing the daily temperature range or spread. On the days in which an abnormal or incomplete anesthetic occurred, an arrow indicates the rise or fall of the maximum temperature from that of the preceding day. The number of degrees appears above the arrow.

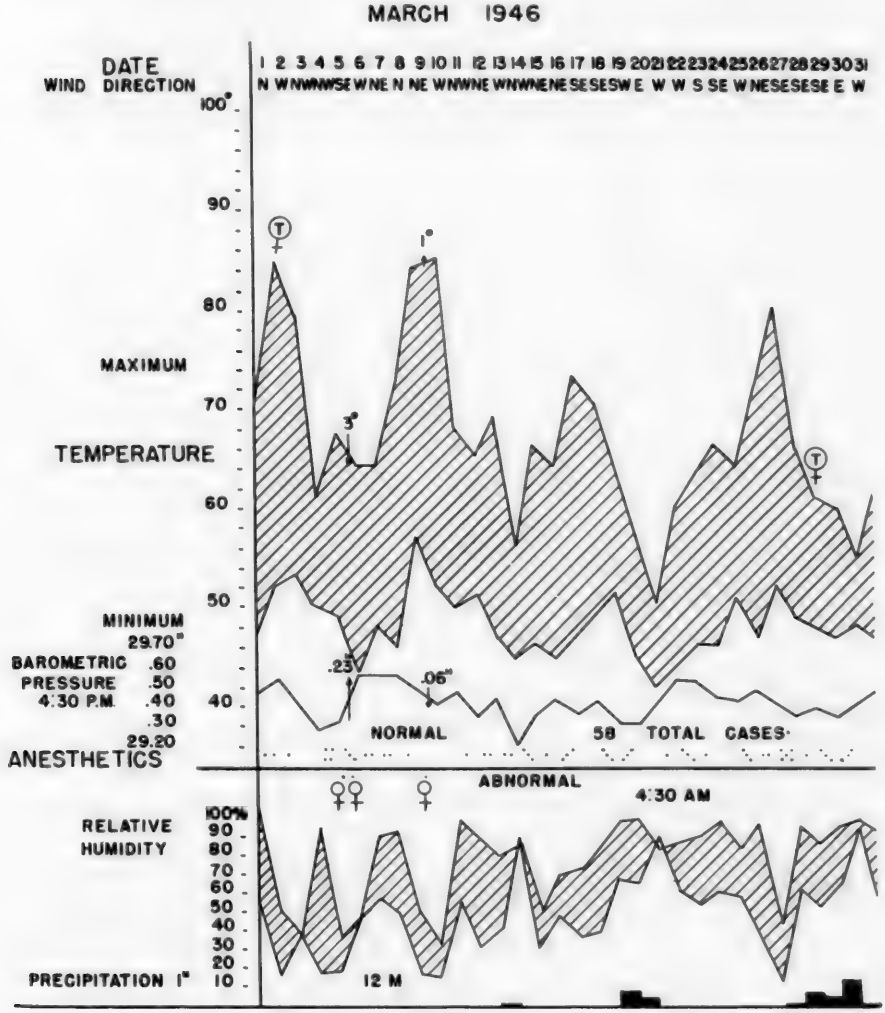

Fig. 1.

Toxic manifestations are indicated by a "T" in the sex symbol on the date of occurrence above the maximum temperature curve.

The daily barometric pressure taken at 4:30 P.M. appears immediately below. The rise (↑) or fall (↓) deviation from the reading of the preceding day at the same hour is indicated in inches. N.C. indicates no change.

Both the normal (above the line) and abnormal (below the line) anesthetic reactions are shown next, with the sex of the abnormal anesthetic cases indicated.

Below these anesthetic recordings are the daily relative humidity (per cent of saturation of the atmosphere) readings taken at 4:30 A.M. and at 12 noon. In the beginning a 4:00 P.M. reading was also included, but it was soon noted that the deviation from the noon reading was so slight that it was discontinued.

At the bottom the precipitation in inches is recorded by the black blocks.

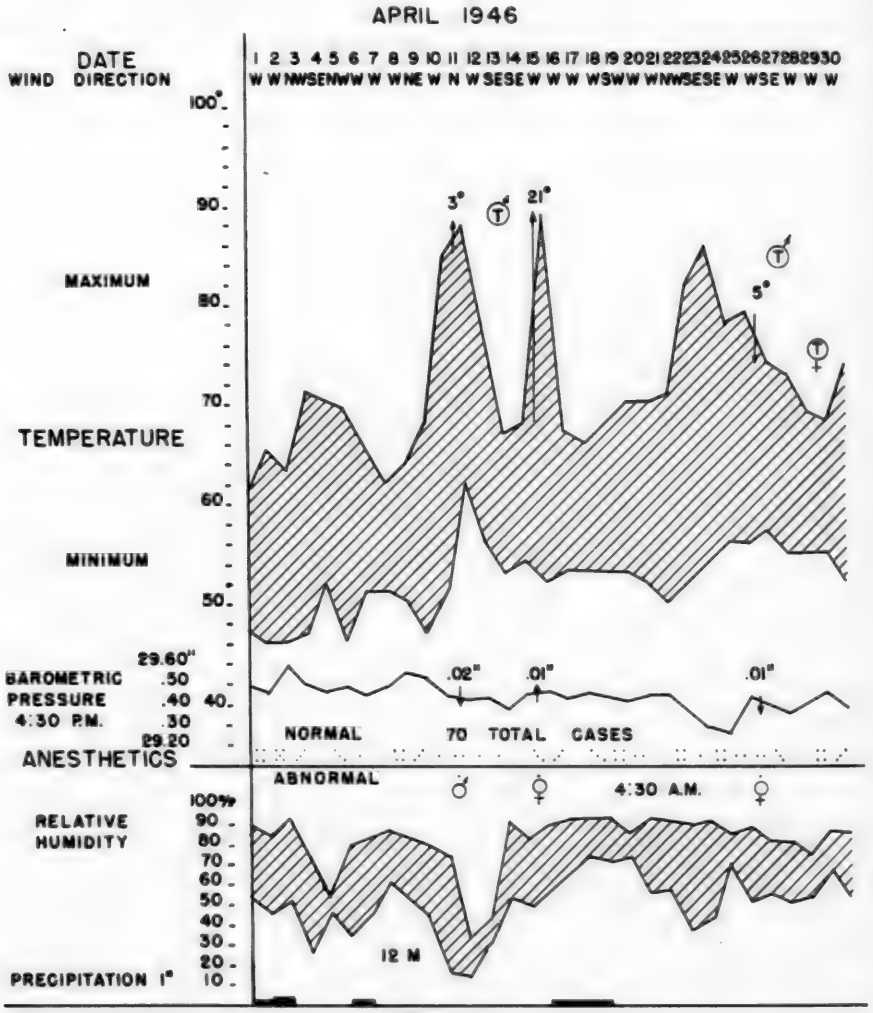

Fig. 2.

OBSERVATIONS

Twenty-four monthly graphs record the daily changes by months in all observed data. In addition, there appear case histories of all instances where toxic reactions or abnormal anesthetics occurred. For illustration the graphs of two consecutive months (Figs. 1 and 2) have been selected.

The toxic manifestation noted March 2, 1946, is somewhat atypical, but it appeared during a five-degree temperature drop from the third of three successive exaggerated peaks occurring in the preceding six days.

The two incomplete anesthetics noted March 5, 1946, were associated with a small temperature peak following the three exaggerated ones previously noted, but also with an unusual rise in barometric pressure of 0.23 inches.

The subsequent incomplete anesthetics and toxic reactions noted in Figs. 1 and 2 are typical of the relationships observed: the abnormal anesthetics appearing near temperature peaks, most frequently on the rising incline, and the toxic reactions following marked temperature falls.

March 1946

Abnormal or Incomplete Anesthetics

March 5: M. W., 36-year-old woman. Mandibular right third molar. Estimated 90 per cent pulp anesthesia. Patient is nervous type, and two months previously had undergone an hysterectomy. Tuberosity injection (left), 5-2-46, gave ninety minutes of complete pulp anesthesia for operations on several teeth.

March 5: D. C., 42-year-old woman. Left mandibular block gave estimated 85 per cent pulp anesthesia. Second injection of 1 c.c. of solution resulted in complete anesthesia within two minutes. Same area had been successfully anesthetized with one injection preceding day, 3-4-46.

March 9: H. D. R., 30-year-old woman. Left mandibular block, estimated 90 per cent pulp anesthesia.

Toxic Manifestations

March 2: I. R., 50-year-old woman. Left mandibular injection, No. 22 of twenty-four anesthetics administered over thirty-month period. Marked blanching observed which passed within two minutes. All anesthetics have been complete for this patient; this is only instance of toxic reaction.

March 29: M. F., 47-year-old woman, unmarried. Because of reactions such as hot flashes, cauterization was done in August, 1943, to halt menstruation. In January, 1946, had one week of flow. February no flow, and March no flow to this date. Right mandibular block was made. Patient showed moderate blanching, experienced tremor in knees and elbow. Anesthesia was complete. Upon leaving operating room after sixty minutes of uneventful bridge abutment preparations, patient experienced sudden, unexpected menstrual flow of hemorrhage proportions, necessitating assistance of nurse. Remained in bed next day and then had no flow. Patient was again anesthetized 4-5-46, with left mandibular block. No objective or subjective toxic symptoms, but upon arrival home, again experienced unexpected menstrual flow, although not so severe as preceding week. Left mandibular block administered 4-19-46 was uneventful in all respects. Patient again noted 4-29-46. Neoplasm, benign or malignant, has been ruled out by competent medical attendants.

April 1946

Abnormal or Incomplete Anesthetics

April 11: L. L., M.D., 29-year-old man. Left mandibular block, estimated 75 per cent pulp anesthesia. Patient subject to constitutional inadequacies, hypotension, renal stone, resulting in release from active duty with Air Corps (M.C.).

April 15: B. O. M., 55-year-old woman. Left mandibular block 90 per cent pulp anesthesia. This patient has for some years experienced low circulatory efficiency, high susceptibility to respiratory infection.

April 26: A. J., 44-year-old woman. Left mandibular block, estimated 80 per cent pulp anesthesia. Nerve hypertension.

Toxic Manifestations

April 13: W. A. C. R., 38-year-old man. Left tuberosity injection, marked blanching.

April 27: W. A. C. R., 38-year-old man. Left tuberosity injection, marked blanching. From June, 1941, through November, 1944, this patient never displayed any untoward symptoms from injections. All gave complete anesthesia. Following his return from foreign service, the last two of four injections produced the previously stated results. The first two produced borderline objective symptoms. Anesthetics administered to this patient in June, July, and August, 1946 have all been complete and have produced no toxic symptoms whatsoever.

April 29: M. F., 47-year-old woman. Patient noted 3-29-46. Right mandibular block. Complete anesthesia. Slight sensation in knees and elbows, but no other reactions. As of May 19, 1946, there had been no further menstrual flow.

Fifty-six (4 per cent) of the 1,319 consecutive anesthetics were found to be abnormal, or incomplete. Nineteen (1.4 per cent) of the 1,319 anesthetics gave minor toxic reactions. Table I shows that incomplete or abnormal anesthetics occurred most frequently with mandibular injections, and that there was no differentiation among the toxic manifestations as to type of injection.

TABLE I

Of the total anesthetics administered:

7.3% of the mandibulars were incomplete
1.4% of the tuberosities were incomplete
2.2% of the subperiosteals were incomplete

1.2% of the mandibulars gave toxic reactions
1.1% of the tuberosities gave toxic reactions
1.8% of the subperiosteals gave toxic reactions

Table II gives the same data in a slightly different light. It is seen that while 73 per cent of the incomplete anesthetics were in mandibular injections, the percentage of toxic manifestations corresponds fairly closely to the total percentages of each type of injection made.

TABLE II
ANESTHETICS BY TYPE

TYPE	ANESTHETICS		INCOMPLETE		TOXIC REACTIONS	
Mandibular	556	42%	41	73%	7	37%
Tuberosity	269	20%	4	8%	3	16%
Subperiosteal	494	38%	11	19%	9	47%
Total	1,319	100%	56	100%	19	100%

Table III indicates that there is little difference observable according to sex. The percentages of incomplete anesthetics by sex very closely approximate the percentage of the total anesthetics administered by sex, as do those of toxic manifestations.

TABLE III
ANESTHETICS ACCORDING TO SEX

SEX	ANESTHETICS		INCOMPLETE ANESTHESIA		TOXIC REACTIONS	
Men	413	31%	19	33%	7	37%
Women	906	69%	37	67%	12	63%
Total	1,319	100%	56	100%	19	100%

In Fig. 3 comparisons of the two twelve-month periods have been made. The first year, from July, 1945, through June, 1946, is represented in both

graphs by the solid line. The broken line represents the second year from July, 1946, through June, 1947. In the upper portion are plotted the number of abnormal or incomplete anesthetics by months for each of the two years. It will be noted that a comparable number of abnormal anesthetics occurred each year with reasonably close approximation in the total number of anesthetics

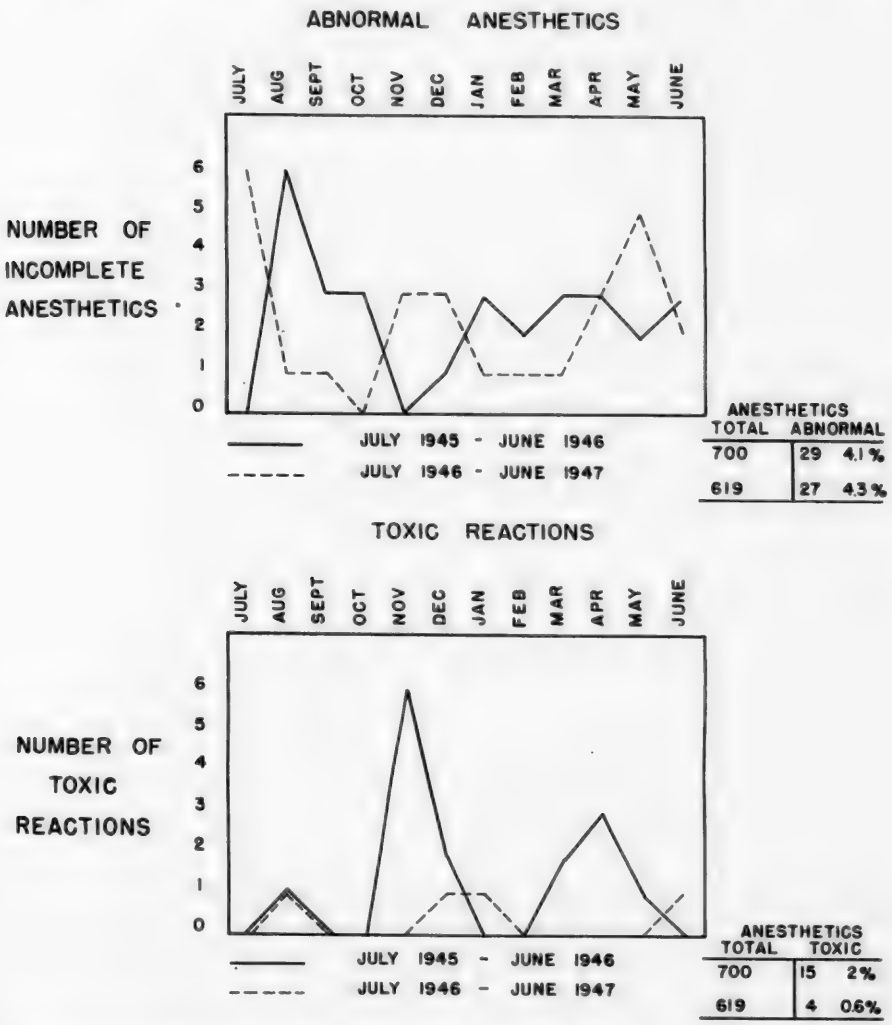
ABNORMAL ANESTHETICS
JULY AUG SEPT OCT NOV DEC JAN FEB MAR APR MAY JUNE
NUMBER OF INCOMPLETE ANESTHETICS
0 1 2 3 4 5 6
JULY 1945 - JUNE 1946
JULY 1946 - JUNE 1947
ANESTHETICS
TOTAL ABNORMAL
700 29 4.1%
619 27 4.3%
TOXIC REACTIONS
JULY AUG SEPT OCT NOV DEC JAN FEB MAR APR MAY JUNE
NUMBER OF TOXIC REACTIONS
0 1 2 3 4 5 6
JULY 1945 - JUNE 1946
JULY 1946 - JUNE 1947
ANESTHETICS
TOTAL TOXIC
700 15 2%
619 4 0.6%

Fig. 3.—Comparison of abnormal anesthetics and toxic reactions for two twelve-month periods.

administered for each year. When allowance is made for the advance or retardation of the seasons it will be seen that the curve for 1946-1947 is very similar to that of the first year of the study. Moving the curve of the second year one month farther along will give a very close parallelism. The graphing of the abnormal anesthetics in this way appears to afford some support to the

previously mentioned seasonal theory. A similar graphing of the number of toxic reactions immediately following is so placed, and on the same scale for comparative purposes. It offers no support for this seasonal concept, since it shows no correlation whatsoever.

Winds blowing from points within the 180 degree sector represented by the directions northwest, west, southwest, south, and southeast are ocean winds. Those from points within the opposite sector are desert winds.

Table IV shows the relation of the daily prevailing wind to anesthetic reactions. Forty-six (82 per cent) of the fifty-six incomplete anesthetics, occurred on days when the prevailing wind was from the ocean, whereas only ten (18 per cent) occurred on days when winds from the desert were prevailing. In the case of the toxic reactions the division is almost equal: nine occurred on days of prevailing ocean winds, and ten on days when desert winds prevailed, for a total of nineteen.

TABLE IV
ABNORMAL ANESTHETICS AND TOXIC REACTIONS RELATED TO DAILY PREVAILING WINDS

PREVAILING WIND	INCOMPLETE ANESTHETICS		TOXIC REACTIONS	
Ocean	46	82%	9	47%
Desert	10	18%	10	53%
Totals	56	100%	19	100%

Figs. 4, 5, 6, and 7 are summaries. They present the averaged conditions preceding incomplete anesthetics and toxic manifestations for a period of thirteen days before the date of occurrence and for the succeeding seven days.

As this investigation progressed, it became evident that the incomplete or abnormal anesthetics were associated with temperature peaks, and most frequently with second or third peaks. The intervening temperature dips are referred to as polar infalls. Furthermore, the toxic manifestations appeared in association with the polar infalls. It is to be noted that the change in temperature is the important factor, not sustained high or sustained low temperatures. In Fig. 4, the totaled plus or minus degrees of departure of the daily mean temperatures from the daily normal temperatures have been graphed. The incomplete anesthetics show a very clear and definite picture. The toxic reactions are less clear, and many more cases would be necessary to determine the exact relationship.

Graphing of the plus or minus departures is standard weather practice. The normals have been established by the Weather Bureau through observations over a period of seventy years. Such a graph for an individual case produces a curve somewhat similar to that produced by graphing the maximum temperatures. In the composite form it affords a very satisfactory means for indicating the temperature relationships.

The temperature range, the difference between the minimum temperature and the maximum temperature, is shown in Fig. 5.* It will be noted that the

*It is necessary to understand the geographic peculiarities of this region in order to evaluate Fig. 5 accurately. This area is close to a large mass of water, the Pacific Ocean. It is obvious, therefore, that there will be relatively few sharp deviations in minimum temperature. Because this is primarily a desert area, the heat from the sun is accumulated and intensified during the day, but for the same reason is dispelled very rapidly at night. Consequently, a wide deviation between the minimum of early morning temperature and the maximum of early afternoon temperature would be anticipated.

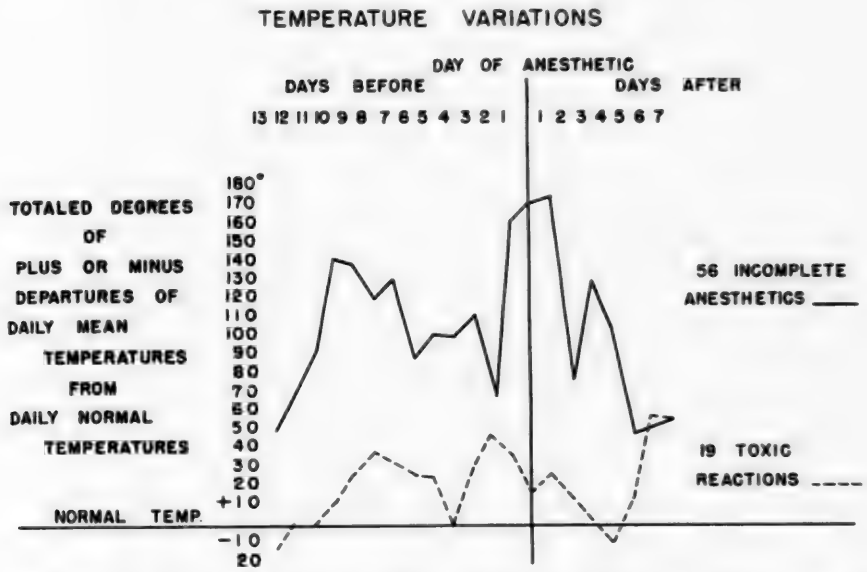

Fig. 4.—Summaries of average conditions preceding incomplete anesthetics and toxic manifestations for a period of thirteen days before the date of occurrence and for the succeeding seven days (1,319 consecutive anesthetics: 56 incomplete anesthetics, 4 per cent; 19 toxic reactions, 1.4 per cent).

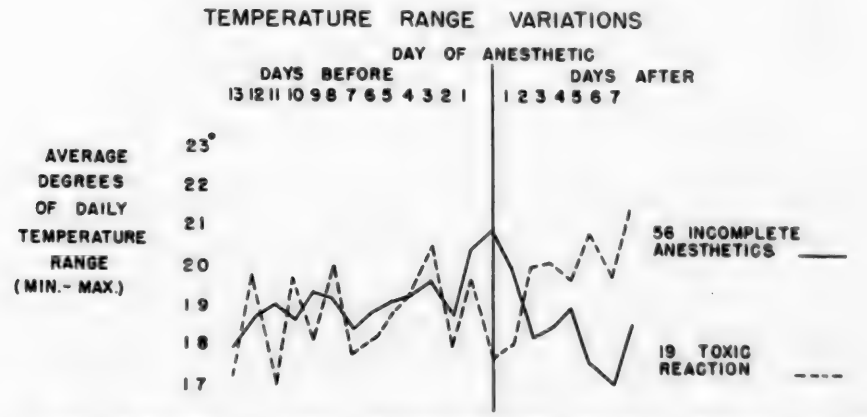

Fig. 5.—Summaries of average conditions preceding incomplete anesthetics and toxic manifestations for a period of thirteen days before the date of occurrence and for the succeeding seven days (1,319 consecutive anesthetics: 56 incomplete anesthetics, 4 per cent; 19 toxic reactions, 1.4 per cent).

curve expressed by the average number of degrees of the daily temperature spread for thirteen days preceding the abnormal or incomplete anesthetics and for the seven days following, is similar to that curve produced by totaling the departures of the daily mean from the daily normal temperatures. Again, the curve shown by a similar charting for the toxic manifestations is less clear, but the alternating large and small ranges in the days preceding the anesthetic reaction are cause for speculation.

As one looks through the monthly graphs, a general drop is apparent in the 4:30 A.M. relative humidity on the days incomplete anesthetics were experienced. The humidity at this hour is normally relatively high, being in the range of 70 per cent to 90 per cent, whereas the relative humidity at 12 noon is comparatively low, except on days of rain. There appears to be a small rise in the 12 noon humidity on these days. Because of the previously mentioned desert characteristics of the area, increased temperatures are usually accompanied by lowered humidity, and in the event of desert winds, which are dry, there is a sharp drop in humidity.

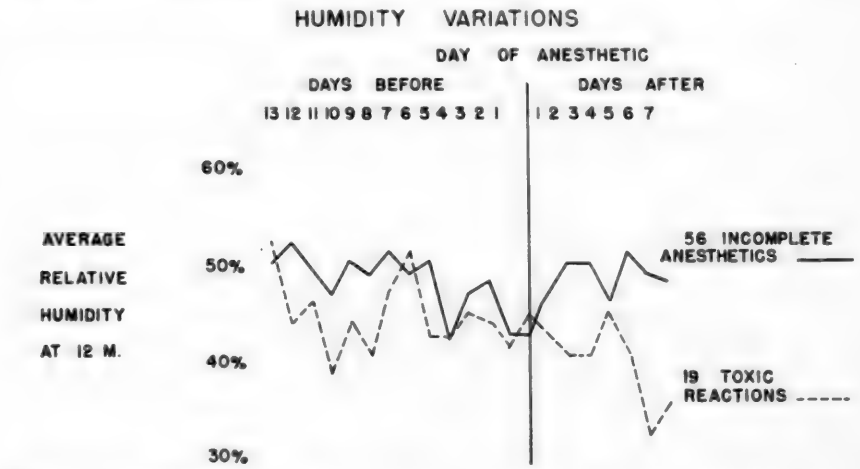

Fig. 6.—Summaries of average conditions preceding incomplete anesthetics and toxic manifestations for a period of thirteen days before the date of occurrence and for the succeeding seven days (1,319 consecutive anesthetics: 56 incomplete anesthetics, 4 per cent; 19 toxic reactions, 1.4 per cent).

A graphing (Fig. 6) of the average daily noon humidity* readings for the thirteen days preceding the anesthetic and the seven days following, shows a distinct drop preceding the day of the incomplete anesthetics, and a slight rise on the day toxic reactions occurred. On the monthly graphs the drop in the 4:30 A.M. humidity is most apparent.

The barometric variations in this area, as stated earlier, are very slight. A graphing of the average variations day by day for the thirteen days preceding

*The noon humidity reading was used as more nearly representing actual conditions at the time of anesthetic administration, and as previously stated the difference between noon and 4:30 P.M. reading is so slight that a better picture is obtained through the use of the meridian reading alone. The curve for incomplete anesthetics in Fig. 6, therefore, is in harmony with that of Fig. 4. Again the toxic manifestations show an inconclusive curve.

an incomplete anesthetic or toxic manifestation, and the seven days following, shows an average deviation of considerably less than one-tenth of one inch. The curve so produced is shown in Fig. 7.

DISCUSSION

In a review of this two-year survey it has become my firm belief that the unusual experiences with local anesthetics are directly related to temperature change. While a possible relationship or correlation may exist with the other weather factors, they are of secondary importance. I recognize the fact that these observations might be considered true for this particular geographic area alone. In other parts of the country, other factors, such as barometric pressure, might well be more important than they are in Southern California.

It has been shown that under environmental temperature changes the distribution of the blood deviates from the normal, becoming predominantly peripheral in distribution with a sudden rise in temperature, or predominantly in the splanchnic beds with a sudden drop in temperature, and that there is a relative anoxia in the tissues affected by reduced blood supply.[2]

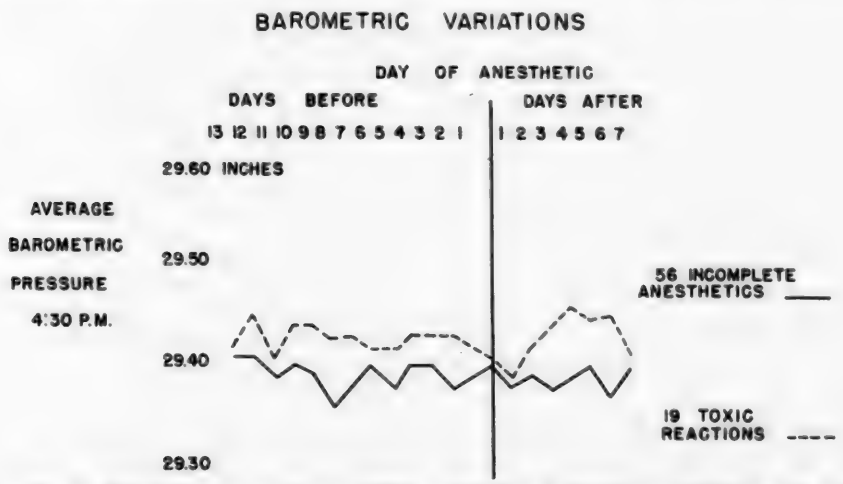

Fig. 7.—Summaries of average conditions preceding incomplete anesthetics and toxic manifestations for a period of thirteen days before the date of occurrence and for the succeeding seven days (1,319 consecutive anesthetics: 56 incomplete anesthetics, 4 per cent; 19 toxic reactions, 1.4 per cent).

Theories of the action of local anesthetics upon the nerve tissue, whether the lipoid theory[13, 14] or the enzymatic theory of Rapp,[15] are predicated upon the neutralization of the acid linkage of the procaine molecule by the buffers of the tissue.

Some years preceding this investigation it had been suggested that alkalinized solutions might overcome the incomplete anesthetics occasionally experienced. To that end freshly alkalinized solutions were used for second injections when an incomplete result was obtained. No improvement in result was noted at this time, and in several instances other anesthetics, such as Monocaine,* and

*Monocaine hydrochloride one and one-half per cent, epinephrine 1:100,000.

in other cases procaine hydrochloride with epinephrine, 1:50,000, were used. It was established clearly in my opinion that the solution used was not the cause of the incomplete anesthetic. For purposes of record, numerous samples from various batches of the solution used in this investigation have been tested and found to average pH 4.35 (colorimetric) with a range of 4.1 to 4.6.

From the evidence presented, it seems very apparent that the anesthetic reactions noted are due to changes in the patient's organic state, caused by temperature change in the weather. The preponderance of incomplete anesthetics among the mandibular injections may be explained by the fact that the injection is made into a relatively less vascular area. With the increased distribution of the blood supply to the peripheral tissues, there would be a lessened buffering action available in the nerve tissue itself. The tuberosity and the subperiosteal injections are made in more vascular areas, and there would be a greater possible modification of the solutions by the blood supply in the area.

The fact that the minor toxic reactions occur approximately equally among all three types of injections makes it appear that the patient's organic state, very possibly the oxidative balance, is the factor which brings the unusual reaction.

SUMMARY

1. Of 1,319 consecutive local anesthetics administered for cavity preparation upon vital teeth, fifty-six (4 per cent) gave incomplete pulp anesthesia or anesthesia of very short duration and occurred in association with temperature peaks.

2. In this series of anesthetics, nineteen (1.4 per cent) gave minor toxic manifestations and occurred in association with temperature dips.

3. The change in temperature appears to be the causative factor for these abnormal anesthetic reactions.

4. Toxic manifestations did not occur in cases of incomplete anesthesia, and vice versa. This may be explained by the fact that it is the temperature drop producing the toxic reactions and the temperature rise producing the incomplete anesthetic.

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THE RELATION BETWEEN LENGTH OF MANDIBLE AND CERTAIN CHARACTERISTICS OF SKULL AND TEETH

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ARIKARA and Hopi skulls were studied to ascertain the correlations between the components of structure and function in the masticatory apparatus. This skeletal material was located, respectively, in the Museum of the University of South Dakota, in Vermillion, South Dakota, and in the Field Museum of Natural History (now the Chicago Museum). The Arikara material came from old village sites in the Missouri River Valley in South Dakota. Most of the material is well preserved.

The Arikara skulls vary in length from 164 mm. to 194 mm., a range of 30 mm., and in width from 130 mm. to 158 millimeters. The mandibles range in length from 88 mm. to 118 mm., a range of 30 millimeters. The bi-condylar diameter varies from 96 mm. to 139 mm., a range of 43 millimeters.

The Hopi skulls and mandibles which were studied were collected by the McCormack Expedition in Arizona in 1901. They came from Old Walpi, Old Mishonovi, and Chukubi. The shortest skull is 143 mm., the longest 180 mm., a range of 37 millimeters. The transverse diameter of the narrowest skull is 125 mm., of the widest 151 mm., a range of 26 millimeters. Many of the teeth had been lost post mortem, and it was not possible to get as extensive data on them as was desired. The longest Hopi mandible was 112 mm., the shortest, 91 mm., a range of 21 millimeters. The greatest bi-condylar diameter was 128 mm., the least, 102 mm., a range of 26 millimeters.

Arikara and Hopi skulls present striking differences in general appearance. The former are more variable in size, and give cephalic indices of dolico-, meso-, and brachycephalic types; the Hopi group contains no dolico-, and only a few mesocephalic skulls. Only six Hopi skulls have a cephalic index as low as 76 to 80, the majority of the skulls being extremely brachycephalic, with the index running up to 102. Pronounced development of the muscles of mastication is indicated by the powerful mandibles, wide rami with mandibular angle approaching that of a right angle, and roughened surfaces for attachment of the masseter and pterygoid muscles. The zygomatic arches are large. The height of the temporal ridges indicates that the temporal muscles were particularly well developed. In most of the skulls the bite was end to end. In each series many of the skulls have wormian bones. The incidence of such skulls is higher among Hopi than among Arikara.

The mean values of the dimensions which were correlated with length of mandible are shown in Table I. The numbers in parentheses indicate number of observations. All measurements are recorded in millimeters (Figs. 1-5).

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TABLE I

MEASUREMENTS OF ARIKARA AND HOPI SKULLS AND MANDIBLES, SHOWING THE MEAN, STANDARD DEVIATION, AND THE COEFFICIENT OF VARIATION

	MEAN				STANDARD DEVIATION				COEFFICIENT OF VARIATION			
	ARIKARA		HOPI		ARIKARA		HOPI		ARIKARA		HOPI	
	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE
Mandible length	107.73 (37)	103.09 (27)	103.62 (21)	100.00 (17)	6.84	7.50	5.39	4.59	6.35	7.28	5.21	4.59
Skull length	179.14 (37)	173.00 (27)	165.48 (21)	157.24 (17)	8.94	4.84	7.59	7.40	4.99	2.80	4.59	4.71
Circumference of temporal muscle	400.79 (37)	360.57 (30)	389.67 (21)	359.00 (17)	24.41	7.94	30.38	16.99	6.09	3.75	7.80	4.73
Height of temporal ridge	93.43 (35)	84.47 (30)	92.26 (21)	86.53 (17)	6.95	5.50	6.62	5.37	7.44	6.51	7.10	6.21
Length of temporal ridge	142.56 (35)	128.35 (30)	130.76 (21)	123.65 (17)	10.07	8.79	8.22	7.11	7.06	6.85	6.29	5.75
Condylo-coronoid diameter	44.90 (24)	41.06 (31)	43.34 (20)	40.74 (17)	2.38	3.06	2.04	2.72	5.30	7.46	4.71	6.67
Length of molars and premolars	41.88 (24)	41.66 (27)	41.31 (16)	40.67 (10)	1.79	2.33	1.10	1.11	4.28	5.60	2.65	2.74
Length of dental arch	52.89 (23)	51.45 (20)	52.49 (11)	51.75 (8)	2.23	3.11	1.47	1.85	4.22	6.04	2.80	3.58
Width of zygomatic arch	19.21 (38)	18.14 (29)	19.43 (21)	17.94 (17)	2.10	2.16	1.68	1.95	10.92	11.91	8.65	10.86
Length of xygomatic arch	41.08 (37)	38.61 (31)	40.31 (21)	38.29 (17)	2.13	2.64	2.00	1.86	5.19	6.84	4.97	4.86
Sagittal diameter of condyle	10.03 (36)	9.02 (31)	10.06 (21)	9.76 (17)	1.30	0.95	1.27	0.64	12.91	10.51	12.66	6.60
Zygomatic arch circumference	108.09 (32)	99.39 (31)	103.90 (21)	99.71 (17)	5.44	5.00	4.48	3.85	5.04	5.03	4.31	3.86
Transverse diameter of condyle	20.43 (36)	18.04 (31)	19.67 (20)	18.18 (17)	3.39	1.58	1.89	1.61	16.61	8.75	9.62	8.88

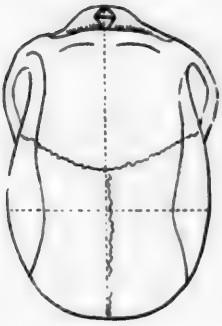

Fig. 1.

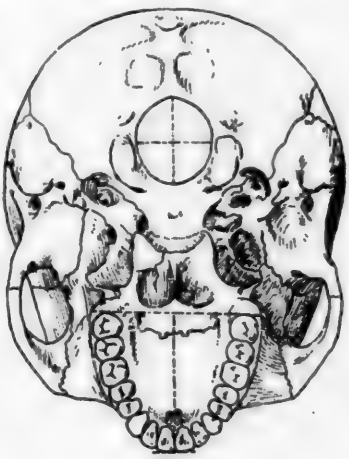

Fig. 2.

Fig. 1.—Skull seen from above (norma verticalis) showing area between zygomatic arch and side of the skull.

Fig. 2.—Aperture at zygomatic arch, basal aspect of skull.

Fig. 3.—Method of taking length of mandible. The length is the distance between parallel planes passing through (1) mentor and (2) posterior border of condyles, when lower margins of mandible are in a horizontal plane (at right angles to the other two planes).

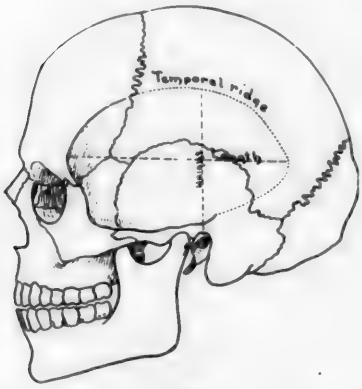

Fig. 4.

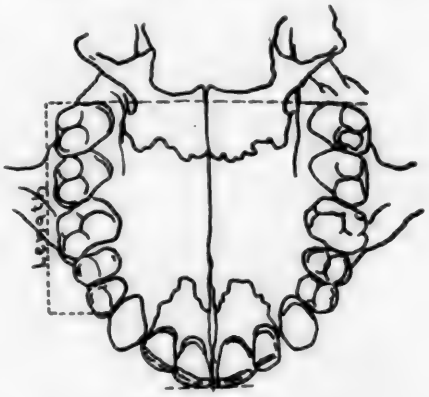

Fig. 5.

Fig. 4.—Side view of the skull showing temporal ridge, enclosed temporal area, height of temporal ridge, and length of temporal ridge.

Fig. 5.—Length of molars and premolars.

TABLE II
VARIABLES WITHOUT REFERENCE TO SEX; CORRELATIONS WITH MANDIBLE LENGTH*

VARIABLES	MEAN		STANDARD DEVIATION		COEFFICIENTS OF VARIATION		COEFFICIENTS OF CORRELATION		PROBABLE ERROR OF COEFFICIENTS OF VARIATION		PROBABLE ERROR OF COEFFICIENTS OF CORRELATION	
	ARIKARA	HOPI	ARIKARA	HOPI	ARIKARA	HOPI	ARIKARA	HOPI	ARIKARA	HOPI	ARIKARA	HOPI
Mandible length	105.73 (64)*	102.00 (38)	7.3	5.4	6.9	5.2			.32	.41		
Skull length	176.59 (64)	161.79 (38)	7.0	8.6	3.9	5.3	.93	.07	.24	.41	.01	.11
Circumference of temporal muscle	382.09 (64)	375.95 (38)	26.2	57.6	6.8	10.5	.41	.12	.41	.82	.07	.11
Height of temporal ridge	89.29 (65)	89.70 (38)	8.2	6.7	9.2	7.5	.82	.27	.55	.58	.03	.10
Length of temporal ridge	136.00 (65)	127.58 (38)	11.8	8.5	8.7	6.7	.46	-.03	.52	.52	.07	.11
Length of molars and premolars	41.78 (51)	41.06 (26)	1.7	1.1	4.0	2.7	.12	.14	.24	.26	.09	.13
Length of dental arch	52.22 (43)	52.18 (18)	2.8	1.7	5.3	3.2	.45	.20	.38	.36	.08	.15
Condylo-coronoid diameter	43.12 (68)	42.15 (37)	3.5	2.1	8.2	4.9	.58	.37	.49	.39	.05	.10
Width of zygomatic arch	18.75 (67)	18.76 (38)	2.2	2.0	11.7	10.4	.43	.09	.80	.81	.07	.11
Sagittal diameter of condyle	9.56 (67)	9.93 (38)	1.3	1.0	13.1	10.6	.31	.12	.78	.82	.07	.11
Transverse diameter of condyle	19.32 (67)	18.98 (37)	3.0	1.9	15.3	10.1	.27	.38	.89	.85	.08	.09
Length of zygomatic arch	39.96 (68)	39.41 (38)	2.7	2.4	6.7	6.1	.38	.15	.39	.47	.07	.11
Zygomatic arch circumference	103.81 (68)	102.03 (38)	6.5	4.7	7.3	4.6	.19	.03	.43	.36	.08	.11

*Numbers in parentheses indicate number of cases.

When the presumed sex of the skulls is not taken into account and each ethnic group is treated as a unit the values listed in Table II are found.

For the Arikara, without regard to sex, the correlation of the circumference of the temporal area with the circumference of zygomatic arch is .73, and for the Hopi it is .46, without regard to sex.

When the skulls are sexed the correlations given in Table III are found.

TABLE III

CORRELATION BETWEEN LENGTH OF MANDIBLE AND VARIOUS SKULL AND MANDIBLE DIMENSIONS

CORRELATION BETWEEN LENGTH OF MANDIBLE AND OTHER VARIABLES	COEFFICIENT OF CORRELATION							
	ARIKARA				HOPI			
	NO.	MALE	NO.	FEMALE	NO.	MALE	NO.	FEMALE
Length of skull	37	.38 ± .09	29	.54 ± .09	21	-.18 ± .14	17	.00 ± .16
Circumference of temporal muscle	34	.60 ± .07	30	.19 ± .12	21	.16 ± .14	17	-.11 ± .16
Height of temporal ridge	35	.58 ± .08	30	.33 ± .11	21	.12 ± .14	17	.19 ± .16
Length of temporal ridge	35	.57 ± .08	30	.24 ± .12	21	-.22 ± .14	17	-.15 ± .16
Condylar coronoid diameter	36	.49 ± .08	31	.38 ± .10	20	-.08 ± .15	17	.42 ± .14
Width of zygomatic arch	38	.43 ± .09	29	.54 ± .09	21	-.03 ± .15	17	-.11 ± .16
Length of molars and premolars	24	.55 ± .10	27	.03 ± .13	16	-.07 ± .17	10	.24 ± .20
Length of dental arch	23	.37 ± .12	20	.46 ± .12	11	-.32 ± .18	8	.59 ± .16
Length of zygomatic arch	37	.27 ± .10	31	.37 ± .10	21	-.12 ± .14	17	.24 ± .15
Sagittal diameter of condyle	36	.18 ± .11	31	.35 ± .11	21	.16 ± .14	17	-.15 ± .16
Zygomatic arch circumference	32	.17 ± .12	31	.45 ± .10	21	.04 ± .15	17	.03 ± .16
Transverse diameter of condyle	36	.16 ± .11	31	.32 ± .11	20	.29 ± .14	17	.29 ± .15

All male and female Arikara correlation coefficients are positive. Hopi male and female skulls yield some positive and some negative correlations, but the coefficients are almost uniformly low in this group. In only two instances is the correlation three times the probable error, and in no instance is it four times the probable error. The highest positive correlations in the Hopi group are in the female skulls. The highest negative correlation is in the male Hopi, namely, the correlation between length of mandible and condylar-coronoid diameter.

The correlations in the Arikara and the Hopi groups, respectively, are very different. In the male Hopi there is a correlation of $-.18 \pm .14$ between length of mandible and length of skull. For the female Hopi the coefficient is $-.00 \pm .16$. In these groups, therefore, the correlation has no statistical significance. In each group the number of individuals is small, and the probable error correspondingly large. In the Hopi group the data indicate an approximation toward zero correlation between length of mandible and length of skull. That the correlation approximates zero is shown also by the fact that when skulls and mandibles are grouped without reference to sex, the correlation is $.07 \pm .11$, the probable error being greater than the coefficient of correlation. Thus the data indicate no correlation between these dimensions in the Hopi group.

The number of cases is smaller in the Hopi group than in the Arikara. (The probable error increases with the decrease in the number of cases, and the reliability of the coefficient of correlation increases as the square of the number of cases.)

In the male Hopi the variables which show positive correlation with mandible length for both male and female skulls are: height of temporal ridge, zygomatic arch circumference, and transverse diameter of condyle.

The variables showing negative correlation with mandibular length for both sexes in the Hopi group are: length of skull, length of temporal ridge, width of zygomatic arch.

Correlations are positive for one sex and negative for the other in the following: circumference of temporal area: positive for male, negative for female; condylar-coronoid diameter; negative for male, positive for female; length of molars and premolars: negative for male, positive for female; length of dental arch: negative for male, positive for female; length of zygomatic arch: negative for male, positive for female; sagittal diameter of condyle: positive for male, negative for female.

Of the twelve variables in the Hopi group showing correlation with mandible length, five are positive for the male and seven are negative. For the female, seven variables are positive and five are negative.

In correlations of the same dimensions without reference to sex, all the variables in the Arikara group show positive correlations between mandible length and the other dimensions. In the unsexed series, the positive values are retained but the size of the correlation is somewhat altered. The Hopi group shows some negative and some positive correlation in the sexed series. In the unsexed series, however, all the variables except one have positive correlations, namely, length of temporal ridge.

Table IV gives the mean sagittal and transverse diameters of the lower molars.

TABLE IV

MEAN SAGITTAL AND TRANSVERSE DIAMETERS OF THE RESPECTIVE LOWER MOLARS

MOLAR	ARIKARA		HOPI	
	MALE	FEMALE	MALE	FEMALE
M_1 sagittal	10.90	10.80	10.90	10.59
M_1 transverse	11.10	10.98	10.70	10.60
M_2 sagittal	10.58	10.10	10.50	10.27
M_2 transverse	10.90	10.60	10.50	9.80
M_3 sagittal	10.65	10.10	10.10	10.10
M_3 transverse	10.57	10.20	10.10	9.70

The molars of males are slightly larger than those of females. The male Arikara has molars slightly larger than the male Hopi. In the two groups there is about the same sagittal and transverse diameter in each molar. Black's measurements indicate that in modern man the mesiodistal diameter exceeds the buccolingual diameters of the lower molars. An inspection of the measurements of fossil man's molars shows that the sagittal or mesiodistal diameter exceeds the transverse or the buccolingual diameter.

The characteristics of the denture of the Arikara and Hopi are: the prevalence of shovel-shaped incisors; the presence of a fifth cusp on the second lower molar; the end-to-end bite of the anterior teeth. Table V shows percentages of shovel-shaped incisors, character of bite, and frequency of occurrence of fifth cusp on lower second molar. In the Hopi group many of the upper anterior teeth either were missing, or were so much abraded from wear that it was impossible to ascertain whether any of the six anterior teeth were shovel shaped. When the finding was not clear it was ruled out. Many of the second molars had been lost post mortem, or had so much attrition that it was difficult to say definitely whether or not a fifth cusp was present. The condition of the material accounts for the small number of cases given in the table.

TABLE V

FREQUENCIES OF SHOVEL-SHAPED INCISORS AND OF END-TO-END BITE

	HOPI						ARIKARA					
	MALES			FEMALES			MALES			FEMALES		
	N*	F†	%‡	N	F	%	N	F	%	N	F	%
Shovel-shaped incisors	22	11	50.0	17	4	23.5	41	22	53.6	31	21	67.7
End-to-end bite	22	17	77.2	17	16	94.1	41	33	90.4	31	23	74.1

*Number of observations.
†Frequency of trait.
‡Percentage frequency.

SUMMARY AND CONCLUSION

The similarity between Arikara and Hopi dentures is striking. Both groups have a high frequency of end-to-end bite, shovel-shaped incisors, and five cusps on the lower second molars. The diameters of the molars and premolars, respectively, of the two groups are practically the same. The length of the dental arch is likewise almost the same, differing less than one mm., although the mean length of the Arikara mandible is somewhat greater than the mean length of the Hopi mandible. These facts suggest that, so far as these two groups are concerned, absolute size of teeth, functional adaptation of lower to upper teeth, and dental pattern are conservative traits among American Indians, and may be used as a criterion for the determination of race characters. The circumference of the temporal area is almost as great in the Hopi group as in the Arikara group, although the Arikara skull is much larger and longer than the Hopi skull. The mean height of the temporal ridge in the Hopi is a little greater than in the Arikara. The width of zygomatic arch is greater in the Hopi than in the Arikara. There is a higher correlation between circumference of temporal muscle attachment and zygomatic arch circumference in the Arikara skull than in the Hopi. The mean condylocoronoid diameter in the Hopi mandible is shorter than in the Arikara mandible by less than 1 mm., although the Hopi mean mandible length is 4.12 mm. less than the mean mandible length of the Arikara. The fulcrum is at the temporomaxillary articulation, the glenoid fossa into which the condyle fits in articulation. The force is applied at the coronoid process through the attachment of the masseter and temporal muscles. Thus the force would be applied closer to the point of

resistance in the Hopi mandible. The force is applied farther from the point of resistance in the Arikara mandible. In the Hopi the mean sagittal diameter of the condyle is greater than in the Arikara. The afore-mentioned differences probably bear on the difference in the correlation coefficients in the two groups.

Both the Arikara and Hopi groups show much attrition on the occlusal surfaces of the teeth, indicating a hard and rough diet. Evidently the biting and grinding mechanisms were well developed. That this is so can be concluded from the end-to-end bite of the anterior teeth, which would also point to a particularly well-developed biting mechanism in which the masseter and internal pterygoid muscles function. The reason that the American Indian has such prominent cheek bones must be attributed to the fact that he used the anterior teeth to a great extent, thereby highly developing the masseter and internal pterygoid muscles, which in turn developed the zygoma and produced the forward and lateral projection of the zygomatic arches.

In conclusion, I wish to stress not so much the specific findings of this investigation, as the method employed in attacking the problem. I realize that in any investigation in which a statistical treatment is used the findings have greater importance if they are based on a larger number of cases, which increases the reliability of the coefficients of correlation. When correlation coefficients are low they are reliable only when based on a large number of cases. In the two groups considered in this study, the Arikara have the larger correlation coefficients; moreover, the correlations are all positive, in both the sexed and unsexed series. In the Hopi group the sexed series give both positive and negative correlations; in the unsexed series, however, all of the Hopi correlations are positive, though low, except in one instance, in which the correlation remains negative.

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THE ABRASIVENESS OF DENTIFRICES AS MEASURED ON THE CERVICAL AREAS OF EXTRACTED TEETH

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THE damage that can be done to dental tissues by dentrifice abrasion was demonstrated by Miller[1] in 1907. The pastes and powders which he employed, and which were presumably in common use at that time, were capable of cutting tooth enamel and their effect on the dentin was even more severe. Following Miller, a number of investigators including Bunting and Rickert,[2] Head,[3] Carney,[4] Wright and Fenske,[5] and Manly,[6, 7] showed a definite wearing away of dentine by abrasive dentifrices. Epstein and Tainter[8] tested the abrasiveness of a number of modern commercial tooth powders, pastes, and liquid dentifrices, using the enamel of the cusps of mandibular molars to measure abrasive effect. They did not use dentin. Although their results may be questioned on the basis of the capacity of their instruments to measure some of the small differentials given, their conclusion that "There are no compounds so unduly abrasive as to be clearly objectionable" is probably true if it is realized that they were considering only results on enamel. From our own observations of the teeth used in the present project we are quite confident that none of the dentifrices, including the most abrasive ones tested, had any observable effect on the enamel. That they did cut into the dentin, and very deeply in some cases, is proved by our results.

It is this effect on the cervical cementum and underlying dentin in patients who have exposure of these tissues with which dentists should be concerned. It has been shown that as little as 1 mm. of such exposure will in time allow dentifrice abrasion to develop. It has been demonstrated that cervical exposure increases with age and that the severity of cervical abrasions is directly proportional to exposure width in individuals with evidence of good oral hygiene.[9]

Rather than being lulled into a sense of security by results obtained by measuring dentifrice abrasion on enamel cusps, it seemed to us that we might well attempt to determine what some of the available dentifrices could do to the often exposed softer tooth tissue.

EXPERIMENTAL PROCEDURE

The abrasion experiments were performed on extracted teeth. The apparatus available was similar to that used by Manly[7] and was illustrated by him. (A motor and counter, neither of which are shown in Manly's illustration, were connected to this brushing machine.) With such a mechanism it is possible to work simultaneously on a series of eight teeth, four on each side.

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Each of the several series of teeth used were as uniform in size as careful selection made possible. In each series, the teeth, numbered one through eight, were respectively an incisor, a premolar, a canine, a premolar, a premolar, a canine, a premolar, and a premolar. Each tooth was mounted in a base of low fusing metal with approximately 2 mm. of labial root area exposed between a metal guard plate and the cervical enamel. All were free from abnormality in the exposed area. Two adjacent sides of the metal base were flattened. This served two purposes: 1. They could be clamped in the brushing machine and after removal and replacement they always were in the same relation to the brush. 2. They could be placed in a holder in a photographic enlarging apparatus, each time in the same position. The teeth were kept wet at all times, excepting for the brief periods in the enlarging apparatus used to secure a profile of the area being brushed.

The brushes were hard bristle nylon of the convex type (West's Miracle Tuft). They were held at a uniform pressure of 75 Gm. on the tooth. The brushing was across the exposed tooth area and each brush had a thin, stainless steel vane set into its end to act as a stirrer for the dentifrice held in the glass container. Brushes were renewed at the end of each two runs. Runs were stopped whenever they had produced a depth of cut approximating 0.3 mm. in the teeth. One back and forth motion was considered as a single stroke. The amount of cut per 100,000 strokes (X) was calculated as follows:

$$\text{Cut in mm.} : X = \text{actual strokes used} : 100{,}000$$

With the lesser abrasive dentifrices the runs were carried out to 100,000 strokes and, in a few cases, over that number. In every case, however, the cut was calculated on the basis of 100,000 strokes. A mechanical counter gave a record of the strokes for each run.

All the dentifrices tested were made into a slurry consisting of 200 Gm. of dentifrice and 400 c.c. of water. This was thoroughly mixed by being shaken in a jar and enough slurry placed in the glass container surrounding each brush and its tooth to cover the tooth. The stainless steel vane in the end of each brush kept the material thoroughly stirred throughout the run.

In testing salt and soda, (equal parts,) and in using Pycopay Powder, both of which contain considerable soluble matter, the question arose as to whether or not the amount of dentifrice going into solution in the slurry would have an appreciable effect on its abrasive action. To determine this, tests were made (1) with a slurry made with water and (2) with a slurry in which the liquid was a saturated solution of the dentifrice. The results with a saturated solution did not show any increased abrasiveness over that with the slurry made with the usual mixture of 200 Gm. of dentifrice to 400 c.c. of water. In fact, the abrasive figures obtained with slurries of dentifrice-saturated solutions were slightly but consistently less than with slurries made with water.

Before a test was started on any tooth, and subsequent to each run, the area to be subjected, or which had been subjected to abrasion, plus a large area of adjacent enamel, was shadowgraphed on 5×7 bromide paper. This was done by means of an enlarging camera equipped with a lens having a fixed

holder for the squared sides of the metal base into which the tooth was fastened. The apparatus was set for an enlargement of twenty. Shadowgraphs following each abrasion test were superimposed on that of the original tooth contour, and by means of transmitted light from a print box the depth of the cut was traced on the graph of the original contour. The contour of the enamel served as a landmark for superimposition. Measurements were made at right angles to the original contour to the deepest point of the cut. In cuts subsequent to the first one, the measurement was from the deepest point of the previous cut to the deepest point of the current cut. This represented the greatest penetration toward the pulp. This method of measurement differs from that used by Manly[7] in that he measured the width of the greatest change in the profile of the cut area. We tried this method, as well as a determination of the profile area by means of a planimeter. A statistical analysis* of the results obtained by each of the three methods on the same series of abrasion cuts indicated that, other things being equal, the methods used seemed to be the one of choice.

Before starting our abrasion tests with the various dentifrices, it was necessary to determine 1, the validity of results obtained by making successive tests on the same series of teeth, and 2, to check Manly's[7] finding, that the use of eight teeth was enough to yield an average which would sufficiently compensate for differences in the results from the individual teeth.

For this purpose we used one series of eight teeth for six successive runs of 25,000 strokes each. The same dentifrice (Colgate's Tooth Powder) was used in all six runs. The numerical results are given in Table I and the successive cuts in each tooth are shown in Fig. 1.

TABLE I

SUCCESSIVE ABRASIONS OF INDIVIDUAL TEETH BY 25,000 STROKES WITH SAME DENTIFRICE.* RESULTS OBTAINED BY MEASURING THE LINEAR DISTANCES AT RIGHT ANGLES TO ORIGINAL TOOTH SURFACE. EACH MEASUREMENT REPRESENTS THE GREATEST ADVANCE TOWARD THE PULP FROM PREVIOUS DEEPEST CUT

	DEPTH OF DENTIN CUT (MM. × 20)							(MM.×20)
TOOTH NO.	RUN NO. 1†	RUN NO. 2	RUN NO. 3	RUN NO. 4	RUN NO. 5	RUN NO. 6	TOTAL	MEAN CUT FOR EACH TOOTH
1	12.0	7.6	8.4	9.3	7.3	6.2	50.8	8.5
2	14.2	8.8	9.8	9.8	8.2	7.5	58.3	9.7
3	6.0	6.0	8.0	7.0	5.3	4.7	37.0	6.1
4	12.0	10.8	13.2	13.0	8.5	6.5	64.0	10.6
5	6.9	5.1	5.8	4.7	11.2	10.3	44.0	7.3
6	8.0	6.3	7.7	7.0	10.0	7.4	46.4	7.7
7	6.0	5.4	5.6	8.0	14.2	11.6	50.8	8.5
8	8.0	7.8	9.7	12.5	10.8	9.2	58.0	9.6
Total	73.1	57.8	68.2	71.3	75.5	63.4	409.3	67.9
Average	9.1	7.2	8.5	8.9	9.4	7.9		8.5

*Colgate Powder.
†Run No. 1 had 25,542 strokes.

From a statistical analysis of the variances shown in Table I, it was found "that the variance due to teeth was significantly larger than the error variance. The variance due to runs was almost equal to the error variance and it seemed

*This analysis and also those on the results given in Tables I and II were made by Dr. Henry B. Mann of the Department of Mathematics, Ohio State University.

fairly safe to assume that the run effect was zero."* Hence, it appeared to be a valid procedure to make successive runs, at least up to six, on the same set of teeth. In no case were tests carried to the extent of exposing the root canal.

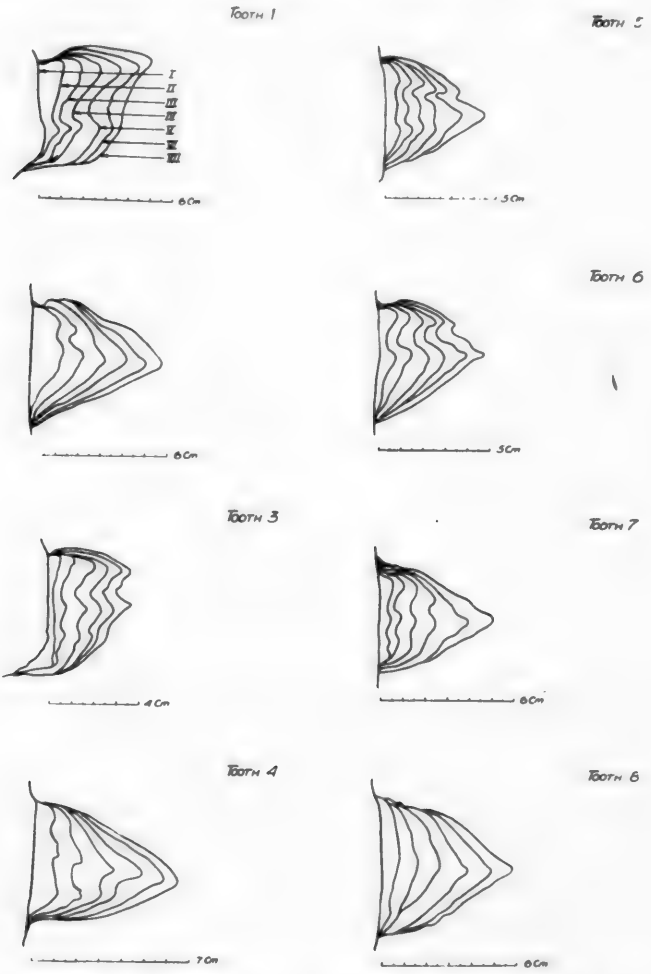

Fig. 1.—Showing the profile of each of six successive cuts in eight teeth by the same dentifrice. This is visual aspect of data in Table 1 and illustrates both variance in individual teeth and in successive runs on same tooth. Scale (in cm.) is given below each profile.

Since the variance due to teeth was greatest, it would have been an advantage to have used more than eight teeth. However, we felt that since this number would be uniform throughout all tests, the results would be comparable and probably accurate enough when considered in the framework of all possible errors.

*Quoted from the report of Dr. Henry B. Mann.

Our first tests were made on nine commercial dentifrices chosen because of their popularity as indicated by sales volume. They were purchased on the open market. The abrasive results from these were quite different. Because we needed for another project a well-graded abrasive series, the Procter and Gamble Co. made up ten experimental paste dentifrices in an effort to fill in the series. These experimental dentifrices, in some cases, varied only in the percentage of the same abrasive used. Under actual test they did not always fall into place on the percentage of abrasive relationship.

As an example of this, dentifrice 4660 contained only 10 per cent of dicalcium phosphate, A.D.R., while dentifrice 4780 contained 52 per cent of the same abrasive. Yet in actual test on teeth the latter proved less abrasive. Again in the case of 4770 with 42 per cent of tricalcium phosphate and 4680 with 52 per cent of the same material, the 4770 was more abrasive. This would seem to throw some doubt on the feasibility of judging the abrasive effect of a dentifrice against cementum and dentin on any basis excepting the results of its use on cementum and dentin.

In addition to the nine commercial and ten experimental dentifrices, we included water (as the probably least abrasive substance) and also equal parts of sodium chloride and baking soda. The entire series of twenty-one tests covered the period December, 1944, to November, 1945.

The results of the abrasive tests are given in Table II.

TABLE II
RESULTS OF ABRASION TESTS

DENTIFRICE	DENTIN CUT (MM. PER 100,000 STROKES)
Water	0.006 ± .0056
4699 B	0.12 ± .0200
4780	0.24 ± .0380
4781	0.26 ± .0405
Colgate Paste	0.31 ± .0334
Salt and soda	0.31 ± .0479
4911 B	0.41 ± .0316
4680 B	0.42 ± .0562
5079	0.48 ± .0767
4660 B	0.56 ± .0915
Pepsodent Paste	0.56 ± .0690
4774	0.61 ± .0245
Pycopay	0.67 ± .0318
4770	0.86 ± .0435
Listerine Paste	0.98 ± .1727
4700 B	1.07 ± .0890
Ipana Paste	1.25 ± .0898
Colgate Powder	2.02 ± .3115
Pepsodent Powder	2.26 ± .188
Calox Powder	3.23 ± .868
Dr. Lyon's Powder	4.48 ± .553

It has been shown by Robinson[10] that the average individual uses twenty-eight single strokes (equivalent of fourteen back and forth strokes as used in this study) in brushing the maxillary anterior area. If we assume that one cleans his teeth twice a day, 365 days per year, then a total of 10,220 cross strokes probably would be applied to the upper right canine tooth in one year's time.

It is of interest to determine what could happen to such a tooth with the commercial dentifrices tested based on an average buccolingual cervical thickness of 7.0 mm. for a right maxillary canine tooth. The period of time necessary to abrade halfway through (3.5 mm.) such a tooth with each of the commercial dentifrices, with salt and baking soda, and with water have been computed on the basis of:

$$\text{Depth of dentin cut (mm.)} : 100{,}000 = 3.5 : X$$

$$\frac{X}{10{,}220} = \text{number of years necessary to cut halfway through a 7.0 mm. canine.}$$

The results of calculations made on this basis are given in Table III.

TABLE III

DENTIFRICE	PERIOD OF YEARS NECESSARY TO CUT HALFWAY THROUGH CERVICAL AREA OF A 7.0 MM. MAXILLARY RIGHT CANINE TOOTH
Dr. Lyon's Tooth Powder	7.6
Calox Tooth Powder	10.6
Pepsodent Tooth Powder	15
Colgate Tooth Powder	17
Ipana Tooth Paste	27
Listerine Tooth Paste	35
Pycopay Tooth Powder	51
Pepsodent Tooth Paste	61
Salt and soda*	110
Colgate Tooth Paste	110
Tap water	6,686 (infinity)

*Equal Parts NaCl (C.P.) and Arm & Hammer Baking Soda.

SUMMARY AND CONCLUSIONS

A series of nine widely used commercial dentifrices, pastes and powders, ten experimental pastes, salt and soda, and water have been tested for abrasive action on the cervical area of extracted teeth. The depth of the abrasive cut per 100,000 cross strokes ranged from almost zero (0.006 mm.) for water through 4.48 mm. for the most abrasive dentifrice tested.

Equal parts of salt and baking soda resulted in an abrasive effect about equal to the least abrasive commercial dental paste tested.

With one exception, the commercial tooth powders tested were markedly more abrasive than were the commercial tooth pastes.

The number of years which would be required for various dentifrices to cut half through a maxillary canine 7 mm. thick has been computed. The values range from infinity for water to approximately 7.6 years for the most abrasive dentifrice tested.

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ACUTE VITAMIN C DEFICIENCY AND THE PERIODONTAL TISSUES

II. The Effect of Acute Vitamin C Deficiency Upon the Response of the Periodontal Tissues of the Guinea Pig to Artificially Induced Inflammation

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IT HAS been noted that in vitamin C deficiency there is an increase in the frequency and severity of local infections.[1-5] This has been attributed to a lowered resistance to infecting agents which results from the generalized deficiency state. Obviously this demonstration of an altered response in local areas resulting from a systemic influence is particularly pertinent to the problem of periodontal disease, a condition in which the interplay of local and systemic factors occupies so significant a role. It seemed desirable, therefore, to determine what the lowered resistance attributed to vitamin C deficiency meant in terms of specific changes in the periodontal tissues. It was felt, too, that some light might be shed upon the nature of periodontal disease by determining the effect of a systemic disturbance such as acute vitamin C deficiency upon the response of the periodontal tissues to local irritation. To accomplish these purposes, a microscopic study was undertaken comparing the changes in vitamin C-deficient and control animals in which the periodontal tissues were subjected to artificially induced irritation.

EXPERIMENTAL PROCEDURE

Twenty-five young adult, vitamin C-deficient and control guinea pigs, described in a previous report,[6] were utilized for this study. After sixteen of the animals had been on a vitamin C-free diet for one month, a 10 per cent solution of silver nitrate was applied to the labial gingival sulcus in the mandibular incisor area in order to induce inflammation in the periodontal region. The silver nitrate was applied with a cotton swab and left in a position for thirty seconds in all the animals, with the exception of two in the vitamin C-deficient and control groups, respectively. All the animals were sacrificed four days after application of the silver nitrate.

It was expected that this procedure would reveal the differences, if any, in the response of the periodontal tissues of vitamin C-deficient and control animals to inflammation induced in the gingival sulcus. For this purpose, attention was focused on the following areas: the labial gingival sulcus, the connective tissue beneath the sulcus, and the underlying alveolar bone.

MICROSCOPIC OBSERVATIONS

Inflammation induced in the gingival sulcus resulted in pocket formation, localized necrosis, and acute inflammation in the underlying tissues of both the

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vitamin C-deficient and the control animals. Although, upon low-power examination, the periodontal pockets thus formed appeared similar in all animals, detailed study of the underlying periodontal tissues revealed that the vitamin C-deficient animals differed markedly from the control animals and presented the following characteristic features: inability to form a peripheral delimiting connective tissue barrier, reduction in inflammatory cells, diminished circulatory response, inhibition of fibroblast formation, and differentiation to form osteoblasts.

The following is a detailed report of the findings:

Nonirritated Periodontal Tissues

Control Animals

The labial gingival sulcus is shallow and formed on one aspect by tooth substance and the other by stratified squamous epithelium (Figs. 1 and 2). A collagenous connective tissue stroma forms the central core of the marginal gingiva and is covered in its labial aspect by keratinized stratified squamous epithelium. The connective tissue which underlies the gingival sulcus and separates it from the labial alveolar bone is comprised of well-formed dense vertical collagenous fibers and elliptical fibrocytes. There is a moderate distribution of capillaries. Occasionally an accumulation of polymorphonuclear leucocytes is seen in the bottom of the sulcus.

The gingival margin of the alveolar bone is comprised of nonlamellated coarsely granular matrix with large lacunae and enclosed spherical osteocytes (Fig. 3). At the periphery of the bone there are cells partially enclosed in connective tissue. The remainder of the labial plate is comprised of nonlamellated bone. Osteoblasts form a prominent peripheral border for the entire bone surface. Osteoclasts are occasionally seen.

The periodontal membrane between the bone and tooth is comprised of vertical and horizontal collagen fibers with many spherical fibrocytes and a moderate distribution of capillaries. The labial covering of the alveolar bone is formed by many layers of closely arranged parallel collagenous fibrils and interspersed fibrocytes.

Vitamin C-Deficient Animals

The labial gingival sulcus is comparable in appearance to that of the control animal (Fig. 4). The connective tissue core of the marginal gingiva is altered in that the outlines of many of the individual collagenous fibrils are obliterated so that there is an appearance of fusion. There is some evidence of edema and the clear staining quality of the collagen is replaced by a poorly staining granular coarseness. Several small scattered areas of hemorrhage are also seen. The connective tissue between the gingival sulcus and underlying bone presents similar changes.

The tip of the bony plate is formed by bone and adjacent degenerated collagenous prebony matrix (Fig. 5). Within the bone many of the osteocyte lacunae are irregularly enlarged and distorted with shrunken fragmented osteocytes. The degenerated collagen matrix contains many shrunken cells.

The bone margin in apposition with the periodontal membrane is irregularly indented with a deeply staining linear border. Occasional multinuclear osteoclasts are seen within the marginal indentations. Many deeply staining flattened polyhedral cells are scattered along the margin. There are no well-formed osteoblasts or pale pink osteoid matrix. The necrotic collagenous matrix observed at the tip of the labial plate extends along the entire labial aspect of the bone.

The collagen in the periodontal membrane appears as shrivelled hyperchromic strands separated by edema and hemorrhage. Small, isolated, poorly staining fragments of degenerated collagen are also seen. The fibrocytes are flattened and hyperchromic.

Fig. 1.

Fig. 2.

Fig. 1.—Control animal. Labial surface of mandibular incisor showing relation of gingival crevice to tooth and underlying bone. Space adjacent to tooth (right) is artefact. (Hematoxylin and eosin; orig. mag. ×24.)

Fig. 2.—Control animal. Detail Fig. 1. Gingival crevice containing a mixture of food, cellular debris, and inflammatory cells. (Hematoxylin and eosin; orig. mag. ×105.)

Fig. 3.

Fig. 4.

Fig. 3.—Control animal. Detail Fig. 1. Tip of labial alveolar bone showing new bone formation with osteoblasts along margin partially enclosed in connective tissue and partially in bone. (Hematoxylin and eosin; orig. mag. ×136.)

Fig. 4.—Vitamin C-deficient animal. Labial surface of mandibular incisor showing gingival crevice. Note unaltered crevice depth. Space adjoining tooth (right) is artefact. (Hematoxylin and eosin; orig. mag. ×66.)

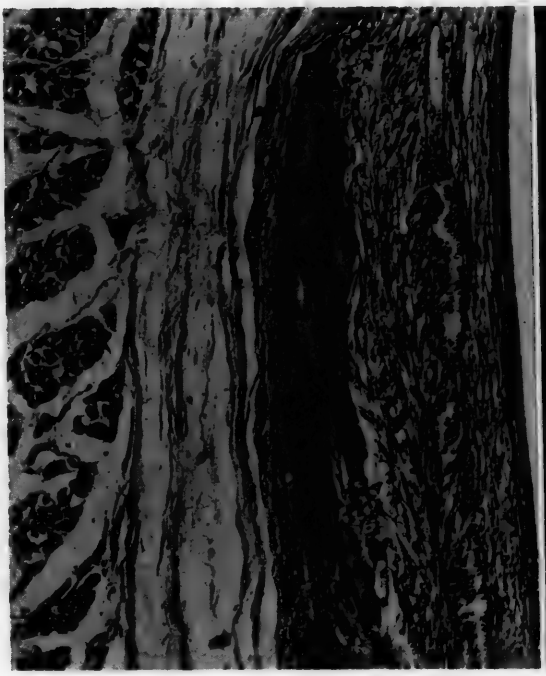

Fig. 5.

Fig. 6.

Fig. 5.—Vitamin C-deficient animal. Labial alveolar plate with strip of degenerated prebony collagenous matrix adjacent to the bone. (Hematoxylin and eosin; orig. mag. ×150.)

Fig. 6.—Control animal. Labial surface, mandibular incisor, showing peridontal pocket with localized area of necrosis at base. (Hematoxylin and eosin; orig. mag. ×96.)

Fig. 7.

Fig. 8.

Fig. 7.—Control animal. Area between base of periodontal pocket and underlying alveolar bone. Note concentration of inflammation beneath base of pocket. (Hematoxylin and eosin; orig. mag. ×60.)

Fig. 8.—Control animal. Detail Fig. 7. Base of periodontal pocket and underlying connective tissue; note dilated blood vessels and dense barrier-like accumulation of polymorphonuclear leucocytes, fibrin, and collagen fibrils. (Hematoxylin and eosin; orig. mag. ×180.)

Periodontal Tissues Subjected to Artificially Induced Inflammation

Control Animals

The gingival sulcus is deepened and presents a spherical localized zone of necrosis at its base (Fig. 6). A mixture of polymorphonuclear lymphocytes, fibrin, and collagen fibers with interspersed dilated engorged capillaries and arterioles forms a dense barrier-like accumulation immediately surrounding the necrotic zone (Figs. 7 and 8). There is some evidence of edema and collagen degeneration in this area, but for the most part the outlines of individual collagen fibrils are clearly discernible.

Beyond the barrier-like accumulation, in the direction of the underlying bone the connective tissue is edematous and hyperemic, and presents a moderate diffuse mixture of polymorphonuclear leucocytes and lymphocytes. In addition, varying degrees of collagen degeneration are seen against a background of well-formed collagen fibrils.

The tip of the underlying labial bony plate is formed by elongated trabeculae of osteoid matrix which blends with the adjacent alveolar bone (Figs. 7 and 9). Contained within the matrix are large spherical cells with prominent nuclei. A multilayered accumulation of connective tissue cells surrounds the osteoid matrix. At the immediate border of the matrix the connective tissue cells are large and spherical in shape. Some areas are seen in which alveolar bone is separated from adjacent inflammation by a dense peripheral border of connective tissue cells (Fig. 10).

Vitamin C-Deficient Animals

The gingival sulcus is deepened and presents a spherical localized zone of necrosis at the base. An area of acute inflammation separates the necrotic zone from the underlying bone (Fig. 11). The appearance of the connective tissue in this area differs considerably from the comparable region in the control animal. There is no suggestion of a dense barrier-like accumulation bordering the necrotic zone (Fig. 12). Many polymorphonuclear leucocytes are dispersed throughout the area but are less numerous than in the control animals. Although there are many capillaries they are not engorged but instead are, for the most part, collapsed and barely detectable. The entire area is edematous.

Collagen degeneration is more severe than that seen in the normal animals. In addition, it is generalized and not limited to the zone immediately bordering the localized area of necrosis at the base of the periodontal pocket. The collagen appears as clumped, hyperchromic, poorly differentiated masses, with internal fibrillar outlines obliterated. Prominent amorphous strands of eosin staining degenerated collagen are also seen.

The tip of the labial plate is formed by a mass of necrotic collagen within which are large lacunae with enclosed hyperchromic nuclei (Fig. 13). The bone adjacent to the collagen is osteoporotic with enlarged lacunae and eroded margins. Amorphous remnants of osteocytes are seen within the lacunae. Although isolated hyperchromic nuclei are present adjacent to the bone, there is no pale staining osteoid matrix or well-formed osteoblasts. The connective tissue surrounding the bone is edematous with degenerated fibers of collagen and hyperchromic connective tissue nuclei (Fig. 14). An accumulation of connective tissue cells comparable to that which surrounds the bone in the control animals is not seen.

Fig. 9.

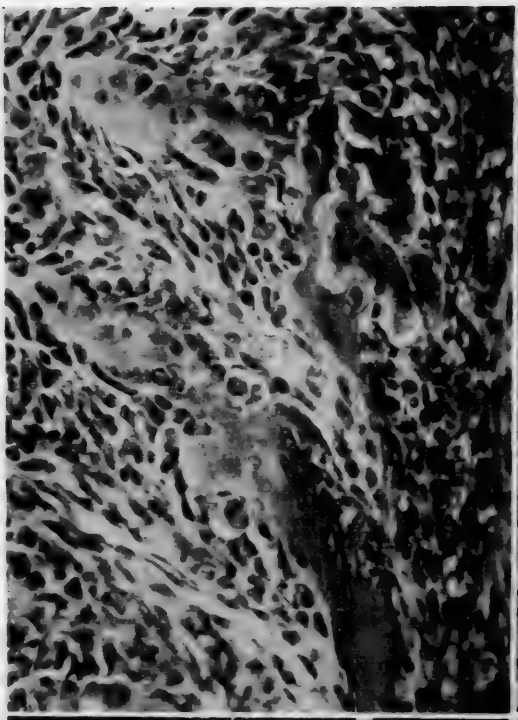

Fig. 10.

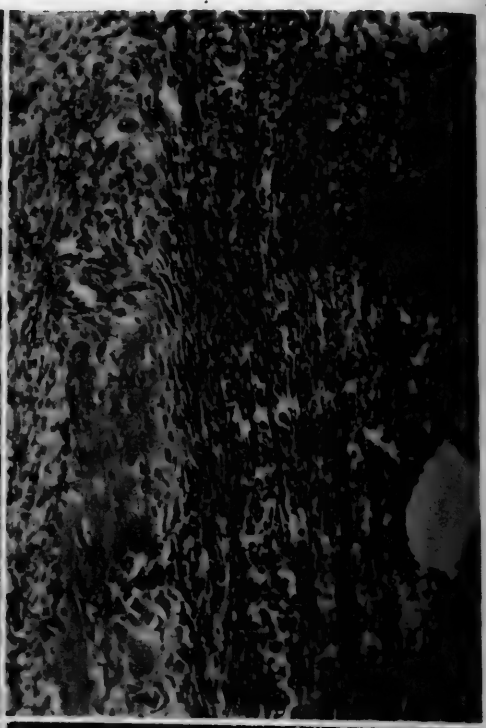

Fig. 11.

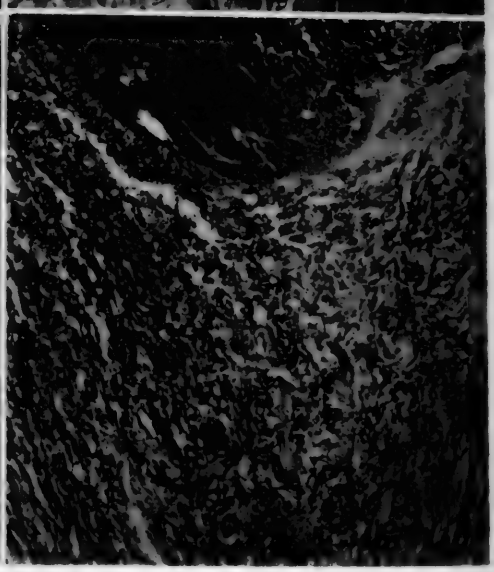

Fig. 12.

Fig. 9.—Control animal. Detail Fig. 7. Tip of labial plate showing alveolar bone, adjoining new bone formation, and osteoclasts. Note dense accumulation of connective tissue cells in area of new bone formation. (Hematoxylin and eosin; orig. mag. ×380.)

Fig. 10.—Control animal. Tip of labial plate in close relation to inflammation. Note new bone formation and connective tissue cells which separate bone from adjacent inflammation. (Hematoxylin and eosin; orig. mag. ×200.)

Fig. 11.—Vitamin C-deficient animal. Labial surface of mandibular incisor, showing area of necrosis at base of periodontal pocket and underlying connective tissue and bone. Space adjoining tooth (right) is artefact. (Hematoxylin and eosin; orig. mag. ×98.)

Fig. 12.—Vitamin C-deficient animal. Detail Fig. 11. Base of periodontal pocket and underlying connective tissue showing generalized edema and collagen degeneration. Note diffuse distribution of inflammatory cells and absence of conspicuous dilated blood vessels. (Hematoxylin and eosin; orig. mag. ×200.)

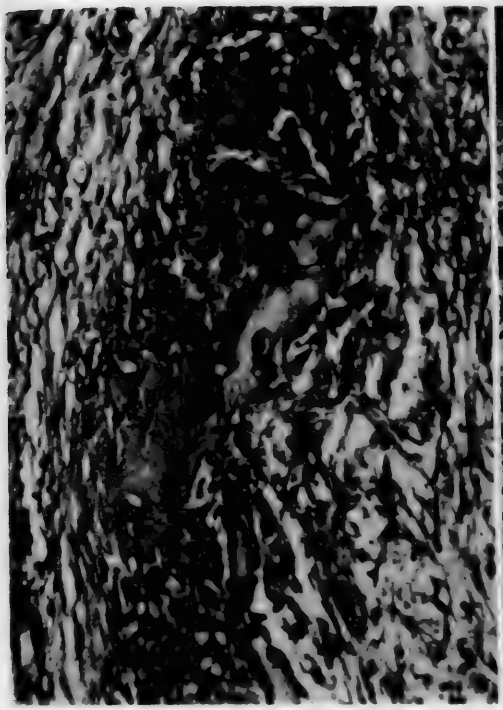

Fig. 13.

Fig. 14.

Fig. 13.—Vitamin C-deficient animal. Detail Fig. 11. Tip of alveolar plate, showing degenerated prebony collagen matrix adjoining osteoporotic alveolar bone. Note absence of osteoblasts and degeneration of surrounding periodontal membrane. (Hematoxylin and eosin; orig. mag. ×380.)

Fig. 14.—Vitamin C-deficient animal. Labial plate in close relation to inflammation. The entire tip of the labial plate is formed by degenerated collagenous prebony matrix. Note absence of peripheral accumulation of connective tissue cells. Compare with Fig. 10. (Hematoxylin and eosin; orig. mag. ×220.)

RECAPITULATION AND DISCUSSION

The changes described are tangible indices of the manner in which acute vitamin C deficiency impairs the ability of the periodontal tissues to limit the injurious effect of local irritation. The microscopic details of this impaired periodontal response are clarified by the following comparison of the findings in the control and vitamin C-deficient animals (A-D):

A. Cellular Infiltration.—In the control group there was a prominent polymorphonuclear infiltration throughout the underlying connective tissue which was concentrated in a barrier-like accumulation at the peripheral zone beneath the necrotic base of the pocket. In the vitamin C-deficient group there were comparatively fewer polymorphonuclear leucocytes and these were scattered diffusely in the underlying tissue.

B. Vascular Response.—In contrast with the numerous engorged capillaries and arterioles observed in the inflamed connective tissue beneath the periodontal pockets in the control group, the vitamin C-deficient animals presented notably fewer vessels. In addition, the blood vessels in the vitamin C-deficient animals were not conspicuously dilated but were collapsed and barely discernible against the background of degenerated collagen.

C. Collagen.—The control animals presented a moderate degree of collagen degeneration associated with inflammation throughout the area between the base of the pockets and the underlying bone. However, well-formed collagen fibrils were concentrated immediately beneath the base of the pocket and in addition were scattered throughout the remaining area. In the vitamin C-deficient animals, throughout the area between the base of the pocket and the underlying bone, collagen degeneration was more severe than in the control group. In addition, there were no well-formed collagen fibrils anywhere in the area.

The findings suggest two facts regarding the collagen degeneration in the vitamin C-deficient animals: First, because of the artificially induced inflammation the collagen degeneration was more severe than that which results from acute vitamin C deficiency alone; and second, the exaggerated severity of the collagen degeneration associated with artificially induced inflammation in the vitamin C-deficient group undoubtedly was due to a superimposed effect of the pre-existent deficiency. The failure to form new collagen fibrils is consistent with the generally accepted impression of acute vitamin C deficiency.

D. Bone.—Osteoblastic activity and new bone matrix were prominent findings at the margin of the alveolar bone beneath the periodontal pockets in the control animals. In the vitamin C-deficient animals there was no new bone formation in this area. Instead, there was degeneration of the collagenous prebony matrix and osteoporosis of previously formed bone. Osteoblasts were rare. The prominent accumulation of connective tissue cells which separated the bony margin from the inflammation and apparently gave rise to the osteoblasts in the control animals was absent in the vitamin C-deficient group. This retardation in the formation and differentiation of connective tissue cells coupled with an inability to form and maintain new bone matrix accounted for the absence of well-formed new bone beneath the periodontal pockets in the vitamin C-deficient animals.

It is apparent that acute vitamin C deficiency alters the response of the periodontal tissues so that the destructive effect of inflammation is accentuated. The exaggerated destruction in the presence of inflammation results partly from an inability to marshal a defensive delimiting reaction to the inflammation and partly from destructive tendencies caused by the deficiency even in the absence of inflammation. Although it was recognized that inflammation and acute vitamin C deficiency, each of itself, exert a destructive effect upon the periodontal tissues, the manner in which the destructive effect of gingival inflammation is influenced by acute vitamin C deficiency had not been shown. The findings in this study reveal first, that there is a lowered resistance to the destructive effect of gingival inflammation in acute vitamin C deficiency, and second, that there are specific histologic features by which this lowered resistance is characterized.

The demonstration of an impaired periodontal response to inflammation in acute vitamin C deficiency and the expression of this impaired response in

specific histological terms are particularly pertinent to the problem of periodontal disease. They serve to reaffirm the interdependence of local and systemic influences in the etiology of periodontal disease and reveal in microscopic detail an example of systemic control upon the effects of gingival inflammation.

Because acute vitamin C deficiency is a potential factor in the etiology of periodontal disease, it is desirable that the nature of its influence be established. Clarification of the interrelationship of acute vitamin C deficiency and gingival inflammation in the causation of gingival disease is a step in this direction. Acute vitamin C deficiency results in hemorrhage and destructive degenerative changes in the gingivae and underlying tissues. It does not, however, cause gingival inflammation.[6] The initiation of gingival inflammation requires the presence of a local irritating factor. The inflammatory changes induced in the periodontal tissues by local irritating factors are different from those which result from acute vitamin C deficiency. In cases of periodontal disease in which both vitamin C deficiency and local irritating factors occur, the ability of the periodontal tissues to withstand the destructive effect of inflammation is impaired. The severity of periodontal destruction is thereby increased. The demonstration of specific histological features which constitute a lowered resistance to the destructive effect of gingival inflammation in acute vitamin C deficiency furthers our understanding of the influence of the deficiency in periodontal disease.

SUMMARY AND CONCLUSIONS

The effect of acute vitamin C deficiency upon the response of the periodontal tissues to artificially induced inflammation was studied in a controlled experiment using twenty-five guinea pigs. Inflammation was induced by an application of a 10 per cent solution of silver nitrate in the gingival sulcus for thirty seconds in vitamin C-deficient and control animals.

Although comparable pocket formation and localized necrosis resulted from artificially induced inflammation in both the vitamin C-deficient and the control animals, associated destructive changes in the underlying tissues were more severe in the vitamin C-deficient group.

Acute vitamin C deficiency altered the response of the periodontal tissues so that the destructive effect of inflammation was accentuated.

Specific histologic features which constitute a lowered resistance to the destructive effect of gingival inflammation in acute vitamin C deficiency were demonstrated.

Whereas a well-formed barrier of collagen fibrils, fibrin, and inflammatory cells separated the artificially induced area of injury from the underlying bone in the control animals, no such barrier occurred in the vitamin C-deficient animals.

Conditions existing in the periodontal area in acute vitamin C deficiency which contribute to a subsequent exaggerated destructive effect of induced in-

flammation include: a tendency toward collagen degeneration and failure to form new collagen, osteoporosis of alveolar bone, and inability to produce well-formed bone matrix.

Factors which follow the introduction of induced inflammation and contribute to the exaggerated destruction of the periodontal tissues in acute vitamin C deficiency include: inability to form a peripheral delimiting connective tissue barrier, reduction in inflammatory cells, diminished vascular response, and inhibition of fibroblast formation and differentiation to form osteoblasts.

The technical assistance of Mrs. Jane Zimmer in preparing the material for this study is acknowledged with gratitude by the author.

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PENICILLIN-IMPREGNATED DENTAL POINTS:

A Clinical and Laboratory Report

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PENICILLIN in some form seems to be a promising medicament against the local infection which underlies root canal therapy. It is recognized that the prevalent organisms causing this infection are of the streptococcus and staphylococcus group, known to be penicillin-sensitive as a whole. It has also long been apparent that the usual medications such as formalin, creosol, phenol, creosote, essential oils, chloramines in water or oil, iodine, thymol or combinations of these and other drugs are all profound tissue irritants and cell toxins. Nevertheless, seemingly successful treatment and saving of infected teeth has been done in the past and is being done now with some of the listed caustics. Undoubtedly the continuation of chronic, painless lesions after treatment has been mistaken often for true healing. Possibly other factors are important besides antisepsis. The body defense may surmount gross local chemical injury if these last factors are properly regarded. Doubtless, mere drainage, cleansing of the root canals with removal of infected contents, proper filling of the canals themselves after treatment, are adequate for the saving of some teeth despite the chemical trauma of irritant antiseptics. No clinical or laboratory data have ever been presented which would tend to evaluate the various possible factors in healing. It is, nevertheless, entirely logical to study an antiseptic which promises uniquely to be nontoxic to the cells of the host, as well as highly bactericidal. Would the use of such a drug reduce the number of visits needed for sterilization? Would it reduce the clinical symptoms of tissue irritation in the course of treatment? Would it cause quickened healing, more rapid disappearance of radiographic shadows around infective roots? Questions such as those have prompted the present study on the value of dry penicillin in the treatment of infected root canals. The method of applying large and predetermined doses deposited on absorbent cotton points was first reported by Buchbinder.[1] The present study, a continuation of this work, was undertaken with the Warner Institute for Therapeutic Research which supplied the points with a potency of 3,000 units of crystalline sodium penicillin (W20-6) and also performed the bacteriological work.

TECHNIC

Root canal cases were selected which involved definite periapical infection; that is all cases presented symptoms of acute or chronic periapical infection, with small or large radiographic shadows. The technic in the operator's chair was standardized: use of rubber dam, painting of the isolated teeth with tincture of iodine 7 per cent, chemical or heat sterilization of all instruments, auto-

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claving of cotton points and pallets, all steps in accepted root canal technic. At the first visit the tooth is isolated with the rubber dam, opened with sterile burs, and a culture is taken in duplicate of the canal fluid, brain-heart infusion broth containing rabbit serum and penicillinase being used. One tube is incubated at the operator's laboratory, the other sent to the Institute for incubation aerobically and anaerobically, plating in blood agar, and for identification of organisms. The root canal is cleansed, washed with penicillin-concentrate solution, finally cement-sealed in the canal, with a point, impregnated with 3,000 units of crystalline sodium penicillin, dry or slightly moistened. At the second visit the technic is repeated. When the cultures have been found to be sterile for at least two successive visits, the canal is filled by a standard method.

PERSISTENCE OF BACTERIOSTASIS IN THE CANAL

To find out for how long a time the penicillin point retains its bacteriostatic effect, the dressing sealed in the cavity at a previous visit was removed and plated on an infusion-agar petri dish seeded with *Staphylococcus aureus* (FDA 209). The dish was incubated and any persistent bacteriostatic action was evidenced by a clear oval zone of inhibition of growth. The experiments were repeated for ten patients, some with several teeth each. The time of retention in the canal was varied from one day to a maximum of nine days. Photographs were made (Figs. 1, 2 and 3) of all petri dishes, and the long axes of inhibition zones were measured and recorded. Control plates were made, standard root canal antiseptics being used. The residual penicillin potency was determined in a few cases by the assaying of removed treatment points.

DISCUSSION OF DATA

(A) *Rapidity of Sterilization.*—Dental points impregnated with 3,000 units of crystalline sodium penicillin were used as a treatment. The present data are based on forty-four cases. Of these: twenty-three cases were sterilized with one treatment, six cases were sterilized with two treatments, three cases were sterilized with three treatments, two cases were sterilized with 4 treatments, two cases were sterilized with five treatments, five cases were sterilized with six treatments, and three cases were not sterilized at all (penicillin-resistant organisms).

Resistant cases omitted, the average treatment time of the remaining forty-one cases was 1.9 visits. The average number of sittings necessary for treating with formocresol or camphorated monochlor-phenol is 8.[2] An average of four has been reported for the number of visits needed to achieve sterility with Azochloramid.[3] The electrophoresis technic has been found to require approximately the same number of visits. Thus, from the data obtained to date, dental points impregnated with 3,000 units of crystalline sodium penicillin are definitely superior in their ability to achieve rapid sterilization. It should be added that unlike azochloramid and electro-sterilization it cannot stain the tooth structure, nor does it require ten to fifteen minutes of extra time in the chair as the latter method does.

(B) *Bactericidal and Bacteriostatic Action.*—Well-defined bacteriostasis was evidenced in most cases tested even three or four days after placing of the penicillin point into the canal and its removal from it. In a few cases even the fluid absorbed by neutral cotton points one or more days after dressing the canal

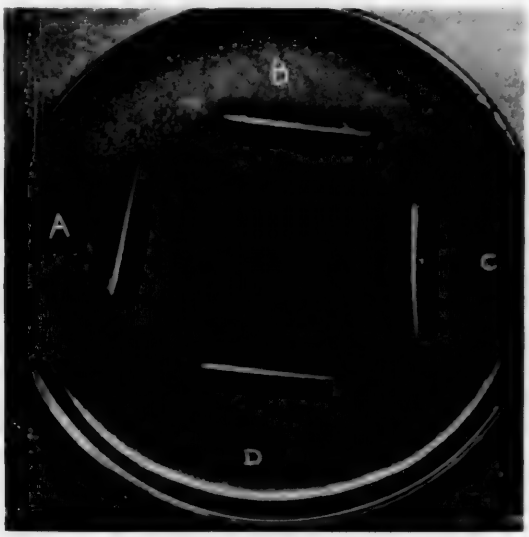

Fig. 1.—Zones of inhibition of freshly saturated dental points with *A*, phenol; *B*, eucalyptol; *C*, eugenol; *D*, creosote.

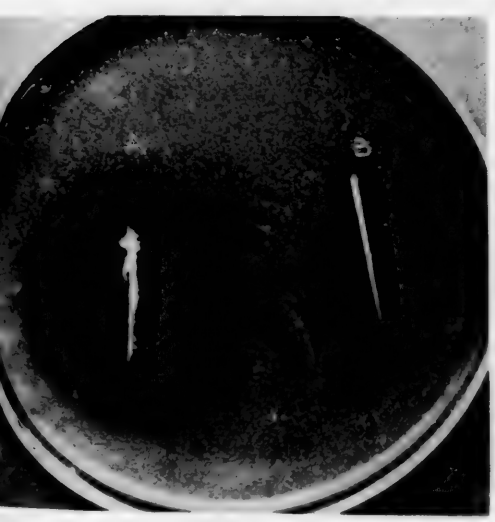

Fig. 2.

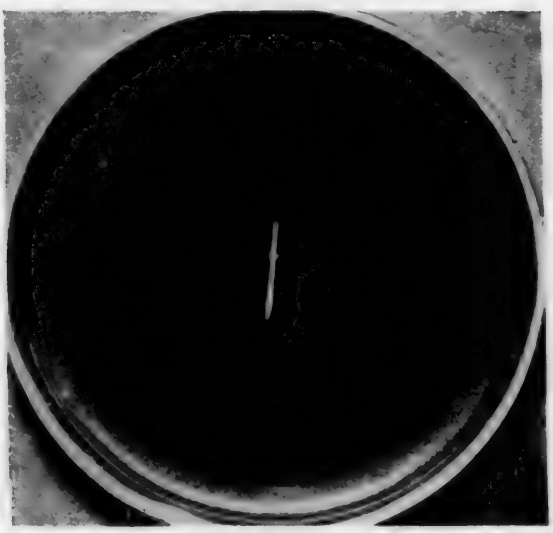

Fig. 3.

Fig. 2.—*A*, zone of inhibition of a penicillin dental point (3000u) removed after forty-eight hours. *B*, neutral dental point showing bacteriostatic effect of root canal fluid absorbed on it after removal of the dressing.

Fig. 3.—Zone of inhibition of a dental point impregnated with 3,000 units of crystalline sodium penicillin.

with a penicillin point was notably bacteriostatic. This indicates a prolonged and active bacteriostatic effect within the canal and tissues. It is interesting to report that the penicillin point sealed in the canal for several days and also the fluid collected on neutral absorbent points had more bacteriostatic effect than of freshly saturated absorbent control points with phenol, eucalyptol, eugenol creosote, and other of the older medicaments. Figs. 1, 2, and 3 illustrate clearly the extent of the bacteriostatic action with penicillin (3,000 units) points after having been sealed in the canal for some days, as well as the comparative effect of fresh phenol, eucalyptol, eugenol, creosote. While it is well recognized that the zone of inhibition is not a good measure of residual penicillin potency in any one dressing point, nevertheless it is unescapable that penicillin (3,000 units) sealed in the canal for several days is more active than the other medications, freshly prepared.* Table I presents the data for all cases tested.

TABLE I.

CASE	TOOTH	TEST NO.	TIME ELAPSED	INHIBITION DIAMETERS (MILLIMETER)	INHIBITION DIAMETERS OF ADDED PTS (MM.)
Mrs. T. B.	1	1	96 hrs.	23×28	
		2	96 hrs.	21×27	
		3	72 hrs.	21×19	
Mrs. K. B.	1	1	24 hrs.	52×48	48×36
		2	72 hrs.	42×35	10×11
		3	7 days	24×27	
Mr. R. K.	1	1	96 hrs.	22×19	
		2	7 days	0	
		3	96 hrs.	33×41	
Mr. R. K.	2	1	96 hrs.	20×24	
		2	7 days	3×10	
		3	72 hrs.	3×17	
Mrs. J. S.	2	1	96 hrs.	12×12	10×10
		2	96 hrs.	8×3	0
Mr. S. M.	1	1	96 hrs.	24×22	
Mr. H. M.	7	1	24 hrs.	45×35	30×40
		2	72 hrs.	25×31	28×24
		3	1 week	0	
Mr. S. S.	7	1	96 hrs.	26×24	
Dr. P. D.	4	1	24 hrs.	38×25	26×20
		2	24 hrs.	28×23	19×15
Mrs. J. W. S.	3	3	48 hrs.	34×27	27×16

BACTERIOLOGICAL STUDIES

The need for bacteriological studies as an adjunct to root canal therapy has been amply demonstrated. Knowledge of root canal bacterial flora is not lacking but it has not been comprehensive nor entirely accurate. With the ad-

*Phenol, eucalyptol, etc.

vent of penicillin into dentistry the need for comprehensive studies on the bacterial flora and sensitivity of these organisms was evident. In connection with the clinical evaluation of penicillin dental points, a bacteriological study was undertaken. All cases were cultured in selective media, the organisms isolated, identified, and tested for penicillin sensitivity.

METHOD AND MATERIALS

The dental points were prepared to contain 3,000 units/point. This was accomplished in the following manner: To vials containing 200,000 units of crystalline sodium penicillin (Pfizer), sixty-six extra fine cotton dental points were added sterilely, and just enough sterile distilled water was added, the volume of water absorbed by these points having been previously determined. The points were dried under vacuum in a dessicator at room temperature for two days. They were then divided into groups of ten, placed in sterile glass vials containing Dryrite, and stored at 5-10° C. and at room temperature. The points were assayed for potency (F. D. A. method) immediately after preparation and after storage for two months at room and at refrigerator temperature 5-10° Centigrade. The potency of these points after preparation and after two months' storage was approximately the same. Stored under dry conditions these points probably have stability of crystalline penicillin.

Technic of Cultural Examination.—For every patient, at each visit, a fresh sterile absorbent cotton point is inserted into the canal after the use of rubber dam, iodizing the field, opening with sterile burs, and removing the previously sealed dressing. The fresh point is inserted to the apex, kept for one minute in the canal, and dropped into a tube of broth media.

Several different media have been employed for cultural examination of root canals. In view of our observations, to be described in detail later, that residual penicillin remains in the root canal for as long as seventy-two hours, we deemed it necessary to use a media containing a penicillin inactivator. We employed brain heart infusion broth (Difco) to which 5 per cent inactivated rabbit serum and penicillinase (Schenley, each 15 ml. of media contained 10 units of penicillinase) were added.

The culture tubes were incubated at 37° C. for forty-eight hours. In addition, 0.1 ml. quantities were plated on blood agar plates, and incubated aerobically and under 10 per cent CO_2 tension. In a number of cases we confirmed the observation made by Grossman with that of glucose aseites medium "that is when growth is present the bacterial colony begins to develop about the tip or surface of the absorbent point and remains there instead of being dispersed throughout the medium."

ORGANISMS ISOLATED AND SENSITIVITY OF TESTS

A total of 216 cultures of root canals were examined and of these seventy-one were positive for one or more organisms. These cultures were taken on forty-four new clinical cases, and on ten clinical cases which had been in progress before the study began. Of these, thirty-five were bacteriologically positive

TABLE II

SENSITIVITY OF ORGANISMS ISOLATED FROM ROOT-CANAL TO PENICILLIN

CLASSIFICATION GROUP	NO. OF STRAINS TESTED	NUMBER OF STRAINS INHIBITED BY ---------- U/ML. PENICILLIN											
		8.0	4.0	2.0	1.0	0.5	0.125	0.06	0.03	0.015	0.007	0.0035	LESS THAN 0.0035
Streptococcus													
Alpha	16		2		2		1		5	4	1	1	
Beta	2								1	1			
Gamma	4	1				3							
Staphylococcus aureus*													
hemolytic	2										1		1
non-hemolytic	5	1			1	1						2	
Staphylococcus albus													
hemolytic	7		2	2	3								
non-hemolytic	15		2	9	1							3	
D. pneumoniae	1							1					
N. catarrhalis	2						2						
N. flavus	5		1				2		1			1	
Micrococcus													
Gram-positive	2							2					
Lactobacillus sp.	1							1					
"Diphtheroids"	5					2		1	1			1	
Fusiform bacillus	1							1					
Totals	68	2	7	11	7	6	5	4	10	5	2	8	1

*_Staphylococcus aureus_ 209 P – Control – Sensitivity – 0.0035.

for one or more organisms. The organisms were isolated and identified (Table II). The most prominent bacteria were: *Streptococcus viridans, Staphylococcus albus,* and *Staphylococcus aureus.* The sensitivity of the organisms was determined by addition of an inoculum of constant size to varying amounts of penicillin (sodium crystalline G). Streptococci and pneumococci were cultured for forty-eight hours in brain heart infusion broth (Difco) with 5 per cent rabbit serum; for the other organisms no serum was used. The inoculum—i. e. 0.5 ml. of this culture diluted to 10^{-3}, in tryptose phosphate broth (Difco)—was added to tubes containing 0.5 ml. broth with decreasing amount of penicillin (1024 to 0.0035 unit per 1 milliliter). The concentration of penicillin required to inhibit growth was determined by direct observation of the test tubes and checked by microscopic examination in hanging drop preparations. In all tests, the strain of *Staphylococcus aureus* (209 P) failed to grow in tube containing 0.0035 unit ml. in forty-eight hours.

The results of the sensitivity tests are summarized in Table I. The sensitivity of the streptococcus and staphylococcus strains varied between 0.0035 and 8.0 units/ml. Of the twenty-two streptococcus strains tested, seventeen were sensitive to 0.5 unit/ml. or less. As a group, the *Staphylococcus albus* strains were less susceptible. Of twenty-two strains tested, only three were sensitive to less than 0.5 unit/ml. The diphtheroids, pneumococcus, and other Gram-positive strains were sensitive to 0.5 unit/ml. or less.

All the Gram-positive organisms isolated and all but one strain of the Neisseria group were found susceptible to penicillin. The Gram-negative organisms which occasionally are isolated from the root canals are for the most part not susceptible, as is to be expected. The incidence of these organisms in root canals is known to be low. Six organisms identified as *E. coli, Klebsiella pneumoniae* (Friedländer's bacillus), *Proteus* sp., *Alcaligenes* sp., *paracolon sp.,* and *Neisseria catarrhalis* (Rough type) were not susceptible to 1024 unit ml. penicillin.

DURATION OF PENICILLIN ACTIVITY OF THE POINTS IN THE ROOT CANAL

Penicillin dental points were placed in root canals for different, definite lengths of time and then were extracted in 1 per cent phosphate buffer solution pH 6.0 and tested for inhibition of *Staphylococcus aureus* (209 P) by the cup plate assay method (F. D. A.).

TABLE III

INHIBITORY ACTION OF PENICILLIN DENTAL POINTS AGAINST *Staphylococcus Aureus*

TIME POINT REMAINED IN CANAL (HOURS)	RESIDUE PENICILLIN IN D. P. UNITS/ML.
6	120.0
18	100.0
24	50.0
48	8.0
72	2.0-0.0

After twenty-four hours in the root canal, the points still had a very good activity, corresponding to 50 units, and after forty-eight hours the activity was sufficiently high to exert a definite bacteriostatic effect (8 units Table III).

The root canal flora contain a rather wide variety of bacterial strains. These are in the main penicillin-sensitive. There is however, an exceedingly wide range of variation as to specific sensitivity within ths group. Some are markedly sensitive; others moderately so. Among the predominant organisms, the *Staphylococcus albus* group is least sensitive. The more resistant strains are readily amenable to the doses of penicillin employed but they might not be to smaller doses.

A small group of penicillin-insensitive strains, primarily Gram-negative organisms, are regularly isolated. Persons infected with these organisms are not amenable to penicillin therapy. They should be recognized and treated with supplementary methods by the alert practitioner. Gram stains of cultures would suffice for recognition. The incidence of this type of root canal infection is small, but it does occur.

CONCLUSIONS

The use of dental points impregnated with 3,000 units of crystalline penicillin appreciably shortens the time needed for sterilization of the canal and tissues. It is important to stress the ease with which the penicillin points may be used in this manner. There is a minimum of effort for the dentist when a large predetermined dose is placed directly into the canal where needed. The addition of a drop or so of sterile water makes available also a highly concentrated penicillin wash in the canal. It is necessary to add here that the root canal cannot be injected with any sizeable volume of fluid. Thus, the injection method proposed by others entails a loss by leakage of most of the injected fluid and the actual retention of only a small and undetermined dosage. Even if injections were feasible it seems obvious that such a method, involving as it does great pressure into an infected area, entails the danger of spreading the infection. In addition, the extra time and effort involved must be considered, as well as the psychological effect of the use of the needle which most patients dread and resent. In brief, even if all things were equal, if equal doses could be placed into the canal by either the point or the syringe, dentists would much prefer the quick, painless, dry or moist dressing to the troublesome needle. As was pointed out, however, the needle method is really impractical and fallacious.

No case was encountered which resulted in swelling or severe pain following the use of penicillin point dosages. Not infrequently teeth sealed with formocresol or other irritants become painful as a result of chemical injury superimposed on bacterial infection.

The points used at present were found to be too long for the average canal. They should be cut to practical length before impregnation and then impregnated with the same or even larger doses of penicillin. The dentist would not have to clip or pack down the overlong point.

Small pellets should be manufactured to act as companions for the point. These could be used easily in canals which are at the start too fine for the use of the regular point. They could also be used to fill the pulp chamber after the penicillin point was placed into the canal. The larger teeth would admit several such pellets easily with very little effort, and the total dose would be greatly enlarged for all canals, particularly the premolar and molar teeth, as well as the large incisor teeth.

No single instance of penicillin allergy, such as rash, swelling or other untoward symptom, was encountered.

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A PERFUSION APPARATUS

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A PERFUSION apparatus is presented. It was designed for the perfusion of the entire heads of experimental animals during studies in which the immediate fixation of the periodontal tissues was a desired objective.

DESCRIPTION

Fig. 1 is a schematic drawing of the apparatus. *A* is the air supply connected to the first chamber, an ordinary wide mouth laboratory jar supplied with a three-hole stopper. *B*, a ground glass straight valve, allows the regulated escape of air from the chamber, thus controlling the pressure passing

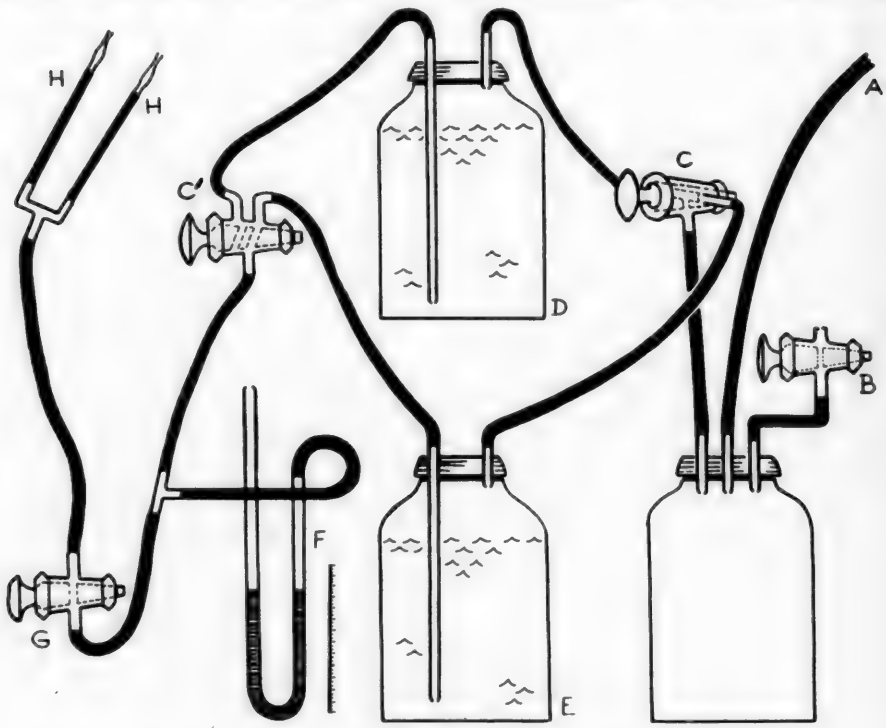

Fig. 1.

through the apparatus. A system of valves, *C* and *C'*, directs the flow through either chamber *D* or *E* which are filled with the perfusing fluids. A manometer, *F*, and a master valve, *G*, are provided for more precise control and operation of the fluid flow through *H*, the leads to the cannulae.

Received for publication Dec. 1, 1947.

PROCEDURE

The apparatus is connected to the ordinary laboratory air supply. Before the air pressure is turned on, the closing of oblique valve, *C'*, and the opening of valve, *G*, will provide the operator with a blood pressure reading directly from the lead vessels. Having the system filled with normal saline will keep the animal's blood from coming in actual contact with the valves and the mercury of the apparatus. Acquiring the blood pressure reading in this fashion enables one to judge the amount of pressure to be maintained during perfusion. The veins draining the region to be perfused are severed at this time.

Ordinarily an isotonic saline solution would be run through first. After a clear flow of fluid is observed from the previously cut veins, the valves may be switched to allow the use of the other chamber's fluid. The apparatus has been successfully used to obtain immediate fixation of tissue for study and with dye solutions for capillary distribution studies. If it is desired to use more than one dye material, this can be done by simply filling the first chamber with the new dye solution while the second chamber is being emptied into the animal.

SUMMARY

The apparatus described is presented as one providing satisfactory equipment for many simple perfusion procedures. It can be constructed from material available in the ordinary laboratory and at a reasonable cost.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Proceedings of Philadelphia Section, December 15, 1947

Compiled by S. LEONARD ROSENTHAL, *Editor of Philadelphia Section*

The Present Status of Dental Caries. *Robert M. Stephan, National Institute of Health, Bethesda, Maryland.* Diet, particularly sugar intake, plays an important role in the caries process. Topical application of urea or its ammonium salts shows inhibitory action on the bacteria. However, any method which relies upon faithful cooperation of the patient in applying a medicament is not suitable for mass control of caries. At the present time, fluorides in the water supply and topically applied to the erupted tooth seem to be the most promising.

Scientific Proceedings of the Philadelphia Section School of Dentistry, Temple University, March 12, 1948*

Solubility of Tooth Substance in Relation to the Composition of Saliva. *Bodil Schmidt-Nielson,** *Swarthmore College.* Saliva contains variable amounts of hydroxyapatite. Tooth enamel is soluble in saliva only when the hydroxyapatite content is below the saturation point. Saliva collected after stimulation is not suitable for this study. Unstimulated or resting saliva is available in sufficient quantity and can be collected in most subjects within ten minutes. One and one-tenth cubic centimeter is sufficient for analysis. Resting saliva is not affected by the time of day or by food ingested. The protein calcium content is constant. Parotid saliva has a more constant hydrogen-ion concentration than does mandibular saliva, usually pH 5.48. It is less saturated with hydroxyapatite than is mandibular saliva.

*Presiding, Paul E. Boyle, Councillor.
**Guest.

PROCEEDINGS OF THE UNIVERSITY OF MICHIGAN SCHOOL OF PUBLIC HEALTH AND SCHOOL OF DENTISTRY INSERVICE TRAINING COURSE FOR THE EVALUATION OF DENTAL CARIES CONTROL TECHNICS

September 8 through 13, 1947

TUESDAY, SEPTEMBER 9

Morning Session—Cont'd

DR. EASLICK.—I think then that we shall call on the second speaker in this morning's symposium. We now must turn immediately to an appraisal of the various control measures which have been recommended to us. The first, and probably the oldest, control method that has been made available to us has come from the periodontists; so we have asked an Indiana periodontist who has been pursuing research work on dental caries to present this first area of caries control. This Indianan is Dr. Maynard K. Hine, Dean of the School of Dentistry at the University of Indiana. The task assigned to him is the appraisal of the value of dental hygiene as a caries control technic. His subject is "Prophylaxis, Toothbrushing, and Home Care of the Mouth as Caries Control Technics." Dr. Hine, I want you to know that we appreciate another neighborly call.

PROPHYLAXIS, TOOTHBRUSHING, AND HOME CARE OF THE MOUTH AS CARIES CONTROL MEASURES

MAYNARD K. HINE, D.D.S., M.S., SCHOOL OF DENTISTRY, UNIVERSITY OF INDIANA, INDIANAPOLIS, IND.

It is generally accepted that dental caries begins on exposed surfaces of the teeth, and that bacteria which accumulate there are involved in the process. Most authorities agree that the carious process is initiated by acid action, although proteolysis has been suggested. In either event, the process may be diagrammed as follows:

(a) Microorganisms + suitable media → harmful products.
(b) Harmful products + susceptible surface → dental caries.

A myriad of other factors, such as rate of acid production, the solubility of enamel, buffering capacity of saliva, must be considered, of course, but it is obvious that the presence of bacteria and suitable media for these bacteria are necessary for the formation of dental caries. Our experience and study have convinced us that the initial step in the carious process is a decalcification of enamel by acid produced by acidogenic bacteria degrading freely fermentable carbohydrate, but a debate of this question is beyond the scope of this paper.

It is also generally accepted that since the carious lesion begins in a relatively small circumscribed area bacteria must be localized, probably by a bacterial plaque, and held in close contact with enamel before the lesion called dental caries is produced. It is natural, therefore, that removal of these bacterial plaques, as well as mechanical removal of the bacterial media, be con-

sidered a method of caries control. The well-known slogan, "A clean tooth does not decay," was coined apparently by Dr. J. Leon Williams years ago,[2] but the idea was stated in other ways as early as 1530.[3]

In 1934 a debate was held before the greater New York meeting on the subject "Resolved: That a clean tooth does not decay, and that mouth cleanliness affords the best-known protection against dental caries."[4] Six well-known dentists took part in this debate, but no conclusions were reached; an editorial in the issue of *Dental Cosmos* containing these papers stated that ". . . we are of the opinion that both sides won this case."[5]

We are convinced that this slogan, "A clean tooth does not decay," is completely correct, if one defines a clean tooth as one which at all times is free of harmful microorganisms and/or free of media for the organisms to utilize. The question to be discussed, as far as I am concerned, is "Can a tooth be kept 'clean' enough, by mechanical means, to prevent or to reduce the incidence of dental caries?"

Although this is an obvious and practical question, few truly scientific studies of this problem have been made. Practically everyone writing on caries control has mentioned the importance of maintaining oral hygiene in inhibiting dental caries, but has given no definite evidence to support the statement. Incidentally, every clinician knows that filthy teeth are not always decayed and that apparently "clean" teeth are often carious. In fact, bacterial plaques, capable of producing an alkaline reaction, may actually protect against dental caries.

Fosdick[6] reported one study on the effect of toothbrushing by twenty caries-active patients immediately after eating. These patients used an antiseptic rinse, antiseptic paraffin, and a toothbrush with a dentifrice. The conclusions were that there was an appreciable decrease in susceptibility of every patient in the test. Doubtless, the reduction in caries activity noted in this experiment was due to mechanical cleansing in part, but the use of medicaments in the experiment makes it difficult to evaluate the efficiency of the cleansing action alone.

Fosdick[7] also reported that he now has approximately 1,000 people brushing their teeth immediately after each ingestion of food. He should have concrete data on this question in another year or so.

In two independent hamster studies, brushed control animals were compared with unbrushed control animals, and in both studies the brushed hamsters showed less dental caries than the unbrushed animals by 20 per cent or more. The two experiments were carried out by Zander[7] and Lazansky.[7] Both reports are definitely preliminary in nature, and this reference may be premature, but these studies do represent an attempt to establish the value of toothbrushing in caries control, and the evidence to date seems promising. Apparently final scientific evidence on the actual value of mechanical cleansing in dental caries control is lacking, but because of the obvious possibilities involved, must be considered. Mechanical cleansing of the teeth may be classified into five methods:

A. Prophylaxis by dentist or hygienist
B. Brushing by patient
C. Mouth rinsing

D. Use of dental floss or toothpicks and various gadgets designed to clean interproximal surfaces
E. Incorporation of detergent foods in the diet

A. Prophylaxis:

Routine scaling and polishing of the teeth by dentists or hygienists at intervals of from three to six months are of positive value in prevention and control of periodontal diseases, but probably have limited value in the prevention of dental caries. Hine and Bibby,[8] in studying the constancy of the oral flora a few years ago, collected bacteria from the distal surface of a lower second molar and a caries-free occlusal fissure sixteen times in eighteen days. Each day a scaler was used, and each time an ample supply of bacteria was found. In another study, we collected material from ten adults every two weeks for twelve weeks. Using the same scaler each time and definite finger rests, attempts were made to collect bacterial plaques from the same areas each time. In every instance it was very easy to obtain bacterial accumulations for study. Obviously, if plaques re-form so rapidly, then removal every three to six months has questionable value. Probably of more importance than mechanical cleansing of the teeth in a prophylaxis is the careful polishing of roughened tooth surfaces and the correction of poor restorations already present. This may conceivably reduce the liability of retention of food particles and bacterial plaques and thus reduce dental caries. No accurate estimate of the efficiency of oral prophylaxis in caries control is available, however.

B. Toothbrushing:

If toothbrushing is to be effective, the following requirements must be fulfilled:

1. The toothbrush must remove the food debris immediately after eating. Stephan[9] and Fosdick[10] have independently demonstrated that acid production occurs almost immediately after food has reached the bacterial plaque. So toothbrushing must be done immediately after eating to be very effective. As Knutson[11] pointed out, such a procedure will probably never receive the sanction of authorities on etiquette.

2. Toothbrushing, to be effective, must be done thoroughly. Casual brushing of exposed surfaces of enamel, already cleansed by excursion of food, will not prevent or reduce interproximal or pit and fissure caries. Gottlieb[2] mentioned that the Charters' method of toothbrushing is the correct one to use "from the caries viewpoint" but it is admittedly a time-consuming, tedious task to brush all surfaces of all teeth.

A study made by Robinson[13] suggests that the average young adult spends about sixty-seven seconds in brushing his teeth. Certainly this is not enough time to allow thorough cleansing of each interproximal surface. Incidentally, Robinson found that the left side of the mouth received an average of three more strokes than the right. Inasmuch as the bilaterality of caries is well estab-

lished (Scott[14]), it would appear that the toothbrush has not played a prominent role in caries control, or at least that the extra strokes on the left are of no value.

Several studies have demonstrated that toothbrushing can reduce the number of bacteria in the oral cavity, but no comments were made regarding the effect of this reduction on dental caries.

Kligler,[15] after his experiments on three patients showed sharp reductions in numbers of bacteria after toothbrushing and mouth rinsing, concluded: "The results indicate, in accordance with prevailing opinion in regard to oral hygiene, that washing teeth before retiring is very desirable, and that rinsing the teeth after each meal is just as expedient a habit to cultivate as washing the hands before a meal." He made no attempt to determine the effect on dental caries of reducing the number of oral bacteria.

Crowley and Rickert[16] developed an interesting method of determining the numbers of bacteria that can be removed from the oral cavity. Their findings, based on application of their method, were that three hours after brushing (twenty-four counts made on four subjects) there was an average decrease on numbers of bacteria of 12 per cent. In a fifth subject (three counts made) there was an increase of 27 per cent in numbers of bacteria removed three hours after the original brushing. Six hours after brushing (twenty-four counts made on four subjects) there was an average decrease of 4 per cent from the number removed at the initial brushing. In the fifth case, there was an increase of 21 per cent above the number removed at the first brushing.

Florestano, Elliott, and Faber[21] made several studies to test the effectiveness of different materials in removing microorganisms from the oral cavity. They found that brushing the teeth with water using Charters' vibratory technic for two minutes, reduced the culturable bacteria that could be collected by 46.7 per cent. Two hours later, however, the count had risen 268.5 per cent.

The experiments mentioned and others indicate that even after thorough brushing of teeth there are still uncounted millions of bacteria remaining in the oral cavity and that more will appear. Therefore, we must conclude that toothbrushing, mouth rinsing, detergent foods, and other mechanical cleansing agents are only partially successful in removing oral bacteria, and hence, at best could do no more than reduce dental caries.

Another possible benefit from brushing the teeth may be that it will make them smoother and prevent the collection of food debris and bacterial plaques. There is no doubt that the toothbrush with a suitable polishing agent in a dentifrice will polish enamel. Theoretically this should reduce dental caries by reducing food retention and perhaps plaque formation. Certainly polishing teeth is esthetically valuable; it increases the brilliance of one's smile. However, even highly polished enamel is still microscopically very uneven, affording excellent areas for bacteria to lodge.

Statistics show that during the past generation there has been an increase in dental caries *and* in the sale of toothbrushes. One must conclude that toothbrushing as commonly practiced is not a good caries control procedure.

C. Mouth Rinse:

Value of a nonmedicated mouth rinse as a mechanical cleansing agent, particularly following toothbrushing, has been mentioned by several writers. Fosdick[6] has suggested it as a simple and fairly unobjectionable method of cleansing the mouth immediately after eating. Theoretically, this should be helpful; actually, no accurate scientific data are available regarding its value.

D. Use of Dental Floss, Toothpicks and Various Gadgets Designed to Clean Interproximal Surfaces:

The same statement can be made for the use of toothpicks, dental tape or floss, and gadgets for interproximal polishing of teeth. They should be of value in isolated instances, where food impaction occurs, but no reports of well-controlled studies are available, so far as I know, which would prove that the incidence of caries was reduced by their use.

E. Detergent Food as Cleansing Agents:

Crowley and Rickert found that after eating there was a reduction of as much as 78 per cent in the numbers of bacteria that could be removed. This finding was also made by Kligler,[15] Wallace,[17] and Knighton,[18] and suggests that mastication of food is the best way to reduce the bacterial count of the oral cavity. In fact, Volker[19] has recently stated that eating of fibrous foods after meals is to be recommended as a method of caries control. Howitt, Fleming, and Simonton,[20] however, fed an inmate of San Quentin Prison several diets, "sticky," detergent, and liquid (by tube), and concluded that artificial cleansing with a toothbrush is many times more effective in reducing the absolute numbers of mouth organisms than the supposed cleansing accomplished by a diet containing detergent foods. However, the fact that areas of teeth exposed to food excursion are so-called areas of immunity, suggests that mechanical cleansing by detergent foods has some value in caries control.

In discussing mechanical cleansing of the teeth, one should consider possible harmful effects that might result. The following should be mentioned:

1. Removal of enamel cuticle undoubtedly results from rigorous cleansing of teeth. No one has proved this to be harmful, however.

2. Abrasion of enamel can occur. However, tests by many, including myself, indicate that while the common dentifrices can wear away cementum and dentin, they are not abrasive enough to affect enamel seriously. The actual amount of enamel lost by toothbrushing is probably not an important factor in dental caries.

3. Mechanical cleansing of the mouth carelessly done may result in gingival recession, exposing cementum which is probably more susceptible to attack by dental caries than enamel (as noted in "senile caries").

4. Will polishing enamel remove or weaken the effects of a fluoride applied topically? If dentists are to continue to recommend topical applications of fluoride solutions, it is necessary to know if mechanical cleansing of the teeth can interfere with its action by removing or altering the surface of enamel. This

seems an important question and although it is presumed that topically applied fluoride reacts with the enamel to produce a more insoluble chemical compound, no definite research data have ever been published to show the actual depth to which fluoride penetrates, and reacts with, the enamel. It is important that the fluorapatite formed in this reaction should be of sufficient depth or hardness to prevent complete removal by prophylaxis or continuous use of a highly abrasive dentifrice.

A study by Phillips[7] of the effect of fluorides upon the hardness of enamel may be summarized as follows: Perfectly flat, highly polished enamel surfaces were prepared on twelve sound molar teeth. Original hardness measurements were then made by means of the Tukon hardness tester, and these teeth immersed in 1:500 tin and sodium fluoride solutions. Following a five-minute immersion period, hardness readings were again made. The percentage increase in hardness of the enamel was 9.9 per cent with tin fluoride and 7.5 per cent with sodium fluoride. The teeth were then brushed for one minute with a slurry of medium grit laboratory pumice by means of a motor-driven Crescent brush. Following the pumice brushing, hardness indentations were again made. There is no particular hardness change noted in the enamel surface following this severe brushing treatment. For example, the enamel gained 9.9 per cent in hardness when immersed in tin fluoride and still showed a gain of 8.5 per cent after the pumice brushing. For sodium fluoride the gain was still 7.1 per cent after brushing. (These figures represent an average of at least five readings in each of six individual teeth.)

A further check on the protective action of the fluorides is to observe the subsequent effect of organic acids on the hardness. Therefore, these same teeth which had been treated with sodium fluoride and then brushed with pumice were immersed for five minutes in acetic acid (pH_4), after which hardness measurements were taken. The final hardness was 3.7 per cent higher than before the use of the fluoride solution; the acid naturally had reduced the hardness somewhat. However, teeth untreated with fluoride and immersed for five minutes in acetic acid showed an actual hardness loss of 6.2 per cent. This experiment indicates the surface was still protected even after one minute of pumice brushing. Contact of tooth surface with 1:500 tin fluoride for twenty-five minutes formed a layer of enamel hard enough or deep enough to remain even after four minutes of brushing with pumice. Further tests are being made on this subject.

It is probably true that rigorous polishing of teeth mechanically will not materially reduce the effectiveness of topical applications of fluoride solutions.

SUMMARY

There is considerable evidence in the literature that mechanical cleansing of teeth will reduce the numbers of bacteria in the mouth and on the teeth. Certainly frequent mechanical cleansing will also reduce the volume of media for microorganisms to utilize. It is probable, therefore, that the cleaner the teeth the less likely they are to decay.

However, it is apparent that the numbers of oral bacteria quickly return to a high level soon after mechanical cleansing, and that it is not possible to remove all oral bacteria by mechanical procedures alone. This coupled with the facts that the cleansing must be done thoroughly and immediately after eating if it is to be effective, makes mechanical cleansing of teeth of limited value in dental caries control. More carefully controlled clinical research must be done to determine the effectiveness of mechanical cleansing.

CONCLUSIONS

1. Widespread mechanical cleansing of the teeth with detergent foods, a toothbrush, a nonmedicated mouth rinse, can be recommended because of its esthetic value, beneficial effects on gingival tissues, and possible interference with dental caries. Routine mechanical cleansing of teeth is apparently not harmful.

2. Because there is no known way of removing mechanically all bacteria and food debris from the teeth for any length of time, mechanical cleansing of the teeth alone cannot be expected to prevent caries.

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DISCUSSION

DR. DONALD A. WALLACE.—I should like to comment on one feature of this paper, the studies on removal of bacteria and other materials from the mouth. The phrase used early in Dr. Hine's paper was "the number of bacteria in the oral cavity."* The number that can be rinsed out or removed mechanically is sometimes taken as a measure of the number in the oral cavity. Actually, as Dr. Hine made clear later in his paper, we don't know how many bacteria there are in the oral cavity. It is impossible to count all of them, or even to remove all of them. When an author refers to the number of the bacteria in the oral cavity, he very generally means the number he was able to remove by some arbitrary procedure.

*In the final paper, Dr. Hine corrected this phrase to conform to Dr. Wallace's suggestion.—Ed.

DR. HINE.—Actually nobody knows how many bacteria there are in the mouth, and I know of no way to find out. We can't kill all of them, and we must admit that there are always innumerable bacteria left in spite of any technic for mechanical cleansing we can use.

DR. BASIL G. BIBBY.—I am a litle cynical on this tooth cleaning business. I wonder whether the problem isn't that when we clean our teeth we never get our cleaning mechanisms—be they patient-applied or dentist-applied—into the areas where caries really originates. Until we can demonstrate that we can actually influence the plaque or the tooth surface accumulations through the depths of the fissures or interproximal spaces, I don't think the problem has too much bearing on what we are trying to answer.

I might mention two studies which cast a little light on the problem. One was by Gavin in Winnipeg, who had a fairly good group of patients in his private practice to whom he gave regular prophylaxis. He showed improved carious condition in those patients. The other was the study by Hyde, carried out in Boston on the inductees in the First Service Command in which he correlated the number of times the patient cleaned his teeth with the amount of dental caries, and got a very nice correlation. Those who cleaned their teeth once a day had less caries than those who cleaned them twice a day, and so on out, with seven neurotic people who were cleaning their teeth five times a day. The correlation was a beautiful straight line correlation: the more tooth cleaning, the more dental caries. However, we have to distinguish between cause and effect; we have to bear in mind that the patients who clean their teeth most may be cleaning them most because they are worried about their teeth, maybe because they have dental caries.

DR. HINE.—I believe that Dr. Bibby agrees that we have no scientific evidence that is adequate to prove that toothbrushing is a good method of caries control. Certainly the report by Gavin (*J. A. D. A.* **20**: 27, 1933) should be mentioned, but he points out that a prophylaxis given periodically has not been proved a good method to control caries.

Until someone can prove that it is in the least degree harmful, I shall continue to recommend that patients continue to brush their teeth vigorously.

DR. JACK M. EARLE.—I believe Dr. Hine mentioned tin and sodium fluoride solutions. Would he tell us what concentration solution he used there?

DR. HINE.—One to 500 solution of tin fluoride and 1:500 solution sodium fluoride were used.

DR. F. D. OSTRANDER.—Was there any special reason why you used tin fluoride?

DR. HINE.—In the studies that were made at Indiana and other places tin fluoride was found to be more effective than sodium fluoride.

DR. PHILIP E. BLACKERBY.—Will Dr. Fosdick or Dr. Stephan express their views regarding the possible effects on caries incidence from the use of mechanical agents, brushing, or irrigation immediately after the ingestion of food?

DR. STEPHAN.—Well, if one could clean the teeth immediately after eating, it would be best. Of course, it takes a considerable length of time to eat in the first place, so that it is hard to see how one could keep them clean as he goes along eating a meal, but if one could do it, I think, as Dr. Hine suggested, the chances are that it would only be partially effective in cleaning the different surfaces of the teeth. As to whether that would really produce the equivalent partial reduction of caries, I don't think we have any evidence at the present time. Certainly since the areas that can be cleaned by our regular prophylactic methods are not the areas susceptible to caries, as Dr. Bibby pointed out. I would certainly reserve judgment until it was actually shown with acceptable scientific data that such was the case. So, it seems to me that the burden of the proof would be on anybody who wanted to establish that immediate cleaning was effective. It may be so, but it doesn't seem a practical method so far as I can see.

DR. EASLICK.—I think perhaps Dr. Blackerby had in mind whether it would remove enough of the sugar solution perhaps to prevent caries.

DR. BLACKERBY.—Well, perhaps if we should decide that we are going to continue, as Dr. Hines suggests, to recommend that patients brush their teeth, then is there an optimum time during the day which that should be done? Are we justified in prescribing that the patients use the toothbrush, if they are going to use it, immediately after the meal, or whenever possible, or is any time of the day effective?

DR. STEPHAN.—I assume that neither is effective.

DR. FOSDICK.—I can say that I don't know. However, I would say also, from a theoretical point of view, toothbrushing should be done immediately after eating for it to do any good. Now, of course, we don't know how much good brushing the teeth will do, or how clean it will make a tooth. For instance, back about 1938 one of our students had a problem of finding out how much cleansing brushing the teeth will do. The method of procedure was to brush one tooth for one full minute and then extract the tooth and stain it to see how much "dirt" remained on it. We couldn't actually determine this, but on the mass basis the maximum removed by brushing was 27 per cent. Now, there is some evidence, I believe, to indicate that flushing the mouth out with plain water may be beneficial. It is not good evidence, but combined with the theoretical evidence it is worth while. It may not clean the teeth, but it certainly could dilute various things that would make it effective.

DR. HINE.—I doubt if we can expect patients to brush their teeth adequately enough for brushing to be a method of caries control. If a person takes a bite of candy the figures, that were shown yesterday and previously, indicate that there is an appreciable amount of acid produced within a very few minutes. Assuming for a moment that it would be possible to cleanse every interproximal surface completely, it would have to be done so quickly that it is not practical. For instance, the average meal takes longer than ten minutes to eat, and if a person takes a bite of fermentable carbohydrate, the reaction starts on the enamel before the patient finishes the last cup of coffee. I believe we must admit it is not a practical method of preventing dental caries. I do believe, however, that if patients brush their teeth after every meal, they will remove considerable debris that might, from a quantitative point of view, allow decalcification to continue longer; a mass of food might allow acid to continue to form for several hours. In a patient who is not particularly susceptible to dental caries, with only a few acidogenic organisms, it may be that it would take several hours for the acid to be produced in quantities sufficient to allow a mass decalcification of tooth structure and cause caries. So, at the present time the only statement that we can make is that theoretically it sounds plausible to remove the media from bacteria and as many of the bacteria as possible from tooth surfaces. We should do this as soon as feasible, and hope that a reduction of dental caries will result.

DR. BLACKERBY.—What is the optimum time for brushing the teeth?

DR. HINE.—I think the toothbrushing can well be done at least before retiring and upon rising. For certain patients that need it, it should be done after meals.

DR. R. W. BUNTING.—I have been very much interested in this discussion, but there are one or two basic facts that I think should not be overlooked. We have been disappointed in the fact that we couldn't control dental caries with the toothbrush, but I think that most of us will admit that we know of many very dirty mouths that have no caries, and of many apparently clean mouths that do have caries in abundance. When caries occurs in the dirty mouth isn't it more likely to be rampant, widespread, and involve more areas of the teeth, while in the clean mouth it is more discrete, and much less extensive? So can we not say that although cleanliness of the mouth in general does not determine whether or not caries shall occur, it does influence the degree of caries activity? Now, one point that has not been raised is significant in my opinion. There is one type of caries that is positively amenable to cleansing with a toothbrush, and that is the typical cervical caries which can be stopped by proper toothbrushing. It is the only kind of caries that can be thus controlled, in my opinion.

DR. HINE.—Dr. Bunting's points are good. It has been suggested that perhaps we are not wise in considering dental caries as always the same process, but should begin to evaluate it more critically. We all have noted that certain mouths are attacked by interproximal caries, other patients have gingival caries as the prominent type, and of course many patients have both types. It is perhaps not quite scientific to group all of these different types of dental caries under the same general heading as though they were identical. I believe that we can agree very definitely with Dr. Bunting that gingival caries appears in mouths where there is a great collection of debris around the mouth; whether that can be controlled by careful brushing or not has not yet been scientifically approved. Dr. Bunting states that it is his opinion—and I imagine that it is the opinion of most of the clinicians in the room—that brushing the teeth in a certain way a certain number of minutes for a certain time reduces certain types of caries a certain amount. At present we don't have the facts, as far as I know, with which to give specific facts regarding caries reduction by the use of a toothbrush.

DR. BENJAMIN M. MEDOF.—The doctor spoke in the brushing of the teeth about the possibility of injuring the delicate Nasmyth's membrane. Now, how long after the tooth eruption does the membrane stay on the tooth, and if it does stay on the tooth, does it probably have any effect on the control of caries?

DR. HINE.—I would just as soon pass that question on to an histologist. Apparently it can be worn away rather easily, and probably is not a factor in caries whether present or not. It is probably gone from most of the areas of the mouth that can be brushed. Actually, we have no facts with which to answer your question.

DR. SAMUEL W. CHASE.—I think Dr. Hine answered his question on the enamel cuticle very nicely. It is a transitory thing, and doesn't have much to do with a tooth that had been erupted for any time.

DR. THOMAS J. HILL.—I should like to support Dr. Hine's contention that we should go on advocating the brushing of teeth, and I should like to refer to the old experiment which was conducted in Cleveland at the Marion School where they took a couple of different classes back in '12 or '14 and in a class, or one group of children in this school, had toothbrushing drills every day over a fairly long period of time. Some fifteen or twenty years later Dr. Harry Wilson went out and collected all of these former students that he could to see whether there had been any evidence that value had been received from the brushing of the teeth. They found that during this fifteen or twenty years the incidence of caries in these two groups of people had been identical, but there was one outstanding difference, and that was that the children who were in the class that had been taught to brush their teeth had very much better teeth because they had become mouth conscious and had had fillings and various other dental work done, so that their teeth were in far better condition. So, I think that there is perhaps a psychological aspect to this also. Brushing of the teeth should be advocated.

Dr. Hine has told us that there are many very dirty mouths that do not have significant dental caries. I think it has been pretty well established that the difference in the plaques that occur in those mouths is that those plaques are primarily proteolytic organisms rather than aciduric organisms. Advocates of the Gottlieb theory should explain why this increased number of proteolytic bacteria in these plaques decreases the incidence of caries rather than increases them.

DR. HARRY STRUSSER.—Has the effectiveness of hand polishing with the porte-polisher been compared to the rubber cup in mechanical cleansing?

DR. HINE.—We have no good evidence that proves that the hand-polished tooth is less susceptible to caries than the one that is polished in the routine way. One can admit immediately that a tooth hand-polished by an expert will be smoother and cleaner than one polished in a superficial manner by a rubber cup. Therefore, from a theoretical point of view, the hand-polished tooth should be less likely to decay. I hope that the time will come when some

good clinician will be able to remove the word "theoretic" from my statement. We don't know whether the hand polishing can be done often enough and thoroughly enough to be effective. We can only state that we will admit it will be smoother and cleaner for a short time at least, and have to let it go there.

DR. JOHN W. KNUTSON.—I would like to have Dr. Hine elaborate on the last conclusion, considering time spent in hand polishing and mechanical polishing. Are they the same, or as I would expect, is perhaps five or ten minutes spent in mechanical polishing and perhaps a half hour in hand polishing?

DR. HINE.—My comments were directed toward hand polishing a tooth by a dentist using a porte-polisher very carefully and very tediously, polishing every surface of the teeth, including areas where a revolving brush or revolving cup could not reach. As far as the patient brushing teeth, how long does it take a patient to brush his teeth in order to clean them adequately? In some mouths it can be done in perhaps ninety seconds, in other mouths, where there is a malocclusion, pathologic pockets, we want the patient to spend from three to five minutes brushing the teeth. I know of a few very fastidious patients who spend ten minutes in the morning and ten minutes at night brushing their teeth. It is all a matter of degree, because even after ten minutes of careful brushing, there will be certain areas of the mouth that will still not be thoroughly cleansed. From a practical point of view everyone in this room probably should spend from two to four minutes and then he can say, "Well, I have done about as well as could be expected." Does that answer your question satisfactorily?

DR. KNUTSON.—Not quite. I was referring to the polishing by the dentist, or the dental hygienist in the office. I understood your statement to say that the hand polishing, porte polishing, was much more effective than the mechanical polishing by a rubber cup. Now in your conclusion did you take into account the difference in the time involved?

DR. HINE.—No, sir. I think that the hand polishing will be accepted by most as being the most effective for interproximal areas and root surfaces, particularly in pockets. I believe at the same time that the average clinician will not feel justified in spending the time involved to do that. Assuming the rubber cup would be, shall we say, 75 per cent effective, the other 25 per cent would take an extra twenty or thirty minutes with hand polishing.

DR. BIBBY.—One point might be added; Dr. Brudevold has been exposing a standard window on a tooth surface and then submerging the tooth in acid, analyzing the tooth from the amount of tooth substances lost. Taking this in successive immersions in acid he finds that in the first exposure to acid the solubility is considerably less than in all subsequent exposures to acid. If, however, he does clean the tooth surface with an ordinary prophylactic procedure using a brush and pumice, then his initial exposure to acid has the same solubility as in the deeper surfaces of the enamel, suggesting that by that procedure of polishing one can remove what seems to be an external and very thin layer of added resistance to acid on the outside of the tooth.

DR. ROBINSON.—Do you infer by that, that some enamel is removed or does polishing remove some sort of a cuticular structure?

DR. BIBBY.—I don't know. I have a suspicion we should regard the change from the enamel plaque into an inorganic enamel not as a sharp boundary, but as a transition in which one finds progressive amounts of inorganic material by proceeding toward the enamel; it takes a harder cleaning to get this outside surface off. So, I assume it is something at least partly calcified. It is, however, a very thin layer. Getting back to Dr. Hine's experiments with fluoride, we find that the first immersion in acid apparently erases the fluoride effect, which is a little different result from what he obtained.

DR. ALLEN O. GRUEBBEL.—I should like to ask Dr. Bibby a question based on what he just said. Might a thorough cleansing of the tooth therefore encourage a carious lesion?

DR. BIBBY.—I have no personal knowledge of that, but there are two reports in the literature of people who went through life caries-immune to the age of forty or so, went to their dentist, got a real polish, and from then on were highly caries susceptible.

DR. EASLICK.—If you people will please come to order, I can promise you a treat in the last speaker of the symposium this morning.

The next speaker, I suspect, will discuss both the caries process and some of the controls on which he has been pursuing research. It was rather interesting in last night's newspaper to note that the thing that was most intriguing about this conference was the fact that the dentists were going to discuss chewing gum and vitamin K. At least, that was the publicity in the Ann Arbor Daily News. Having heard the next speaker quite recently at Northwestern University where he presented this subject of dental caries most lucidly, I can promise you a very informative forty-five minutes. Dr. Fosdick is professor of chemistry at the School of Dentistry at Northwestern University, and he has been assigned the subject by the committee "The Degradation of Sugars in the Mouth and the Use of Chewing Gum and Vitamin K in the Control of Dental Caries." It is certainly a pleasure, Dr. Fosdick, to have you repay my visit to Northwestern.

THE DEGRADATION OF SUGARS IN THE MOUTH AND THE USE OF CHEWING GUM AND VITAMIN K IN THE CONTROL OF DENTAL CARIES

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Although there is a widespread belief that chewing gum is of prime importance as an etiological factor in dental caries, there is very little factual evidence to substantiate this belief. It has been found by many investigators that the lactobacillus count of individuals under dietary control cannot be substantially reduced unless the chewing of gum is eliminated from their daily routine. Thus, individuals who are otherwise prohibited from ingesting carbohydrates show little or no improvement unless they abstain from chewing gum. On the other hand, there are several factors which should influence the effect of chewing gum which have not as yet been publicized.

Chewing gum consists primarily of gum base, flavors, and fermentable sugars. Insofar as the material is approximately 60 per cent sugar, one would normally expect that the sugar in the gum would be easily available for the bacterial substrate.

In 1942 it was shown that 2-methyl-1, 4-naphthoquinone was an excellent enzyme inhibitor for some of the enzymes involved in the anaerobic degradation of carbohydrates to lactic acid,[1] and hence, if present in the mouth with the carbohydrate, it should prevent the formation of lactic acid. This substance was used in an experiment based upon a modern theory of the etiology of dental caries.[2]

Although the paucity of material concerning the use of enzyme inhibitors and concerning the use of chewing gum in the control of dental caries makes it hazardous to predict the true effects, there is an abundance of theoretical evidence which would indicate certain effects. Furthermore, insofar as experimental evidence has partially substantiated most of the concepts proposed by the theory, it is practical to theorize on the probable effects of other enzyme inhibitors and on the effects of chewing gum.

In 1936[3] it was suggested that the mechanism whereby acids are formed in the mouth from carbohydrate material was identical to the anaerobic degradation of carbohydrate in muscle tissue. Furthermore, it was subsequently shown[4-7] that bacterial metabolism involved the same types of reaction. In view of the foregoing, it was not surprising that the intermediate products of anaerobic degradation of glucose[8] were isolated from fermenting saliva glucose mixtures with no extraneous bacteria present. It was also found at this time that a predominant difference between saliva from caries-free individuals and caries-active individuals was the rate at which these acids would form.[9] The actual rate of acid formation in the mouth was not determined until later when Stephan[10] demonstrated that the reactions were extremely fast and analogous

to that which occurs in muscle tissue during the anaerobic phase of carbohydrate metabolism. It was found that when sugar is placed in caries-susceptible areas in the mouth, it is immediately degraded to lactic acid and other acids and that if the area be isolated from the neutralizing effects of saliva, pH values as low as 4 can be obtained in as short a time as three minutes.[11] Furthermore, it was shown that the speed with which acids are formed in isolated areas in the mouth varies from individual to individual, and also varies within the same mouth. In immune individuals the production of acid was extremely slow. In carious lesions, the interproximal spaces and pits and fissures of susceptible individuals, the process was extremely fast.

It has been shown by many investigators that under ordinary conditions the saliva is a highly buffered mixture which will neutralize relatively large quantities of acids. Furthermore, the acid neutralizing power of the saliva is dependent to a large extent on the diet and the state of nutrition of the individual.[12, 13] From a chemical point of view, the foregoing immediately suggests that there is a competition going on in the mouth between the rate at which acids are formed and the rate at which they are neutralized. Thus, if acids are formed rapidly from ingested carbohydrates, they may or may not attack the teeth, depending upon the rate at which they are neutralized. If they are neutralized before sufficient time has elapsed for dissolution of the teeth to take place, then a carious lesion will not develop. On the other hand, if they are not neutralized or only slowly neutralized, then a carious lesion will form. Thus, whether or not a substance will affect the caries process will depend on the action of (1) the rate of the acid formation, and (2) the rate of acid neutralization.[2] (Fig. 1).

In order for acids to form it is necessary to have present in the mouth a substrate in the form of a fermentable carbohydrate or something that is rapidly converted to fermentable carbohydrate. It is also necessary to have the proper enzyme system present. If either one is lacking, then acids do not form.

In order to have these acids neutralized, it is necessary to have a copious, never-ending flow of highly buffered saliva to all portions of the mouth in which acids are forming. The rate of neutralization, therefore, depends upon a tremendous number of variables.

If we assume a mouth with ideal occlusion, no pits and fissures, and no bacterial plaques, then the rate of neutralization will depend upon the rate of flow of stimulated saliva and the buffering capacity of the saliva. Unfortunately, there is a large difference between the mechanical cleansing ability of the different mouths. Poor contacts, malocclusion, pits and fissures, dental plaques, and dental appliances all contribute to impeding the normal flow of saliva throughout the spaces of the mouth. Thus, it is possible that even though an individual may have a copious flow of stimulated saliva, other local factors consisting of mechanical impediments may defeat this copious flow of saliva and cause areas which the saliva cannot readily reach. All of these variables contribute a definite measure to caries activity or caries susceptibility. We are uninformed, particularly about all of the characteristics and the role of the bacterial plaque.

With these factors in mind, in order to evaluate any process of caries control, it is only necessary to determine how the control measure would fit into the scheme of events that take place during caries production.

From a theoretical point of view, any inhibitor that would block any one of the twelve reactions involved in the degradation of fermentable carbohydrates to lactic acid should prevent the formation of lactic acid, the only requisite being that the enzyme inhibitor must be present in the mouth where the acids are forming concurrently with the substrate or fermentable sugar. From a theoretical point of view, if the enzyme inhibitor is present in sufficient concentration to stop the acid formation, then no caries could result. The difficulties in obtaining this condition are obvious. No known enzyme inhibitor, with the

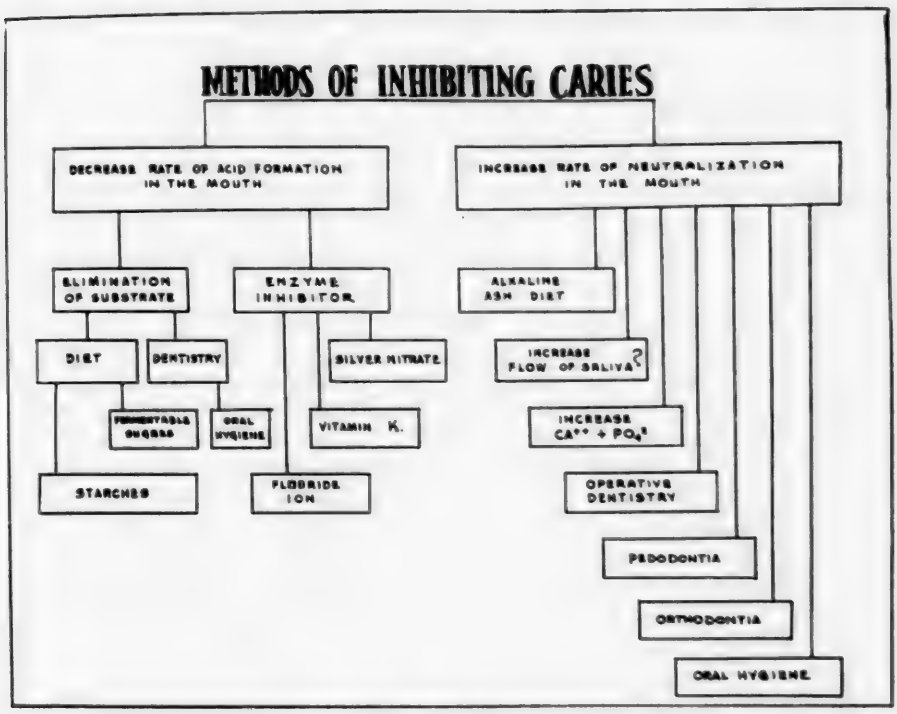

Fig. 1.

exception of the fluoride ion, can be maintained in susceptible areas of the mouth for any appreciable length of time. For this reason, any enzyme inhibitor that will not maintain a concentration in the mouth for appreciable periods of time must be placed there along with the ingested carbohydrates or shortly thereafter. Furthermore, the enzyme inhibitor must be sufficiently nontoxic so that when it is absorbed into the blood stream it will not inhibit the same type of reactions which are so necessary for the conversion of carbohydrates to energy. Whether or not the enzyme inhibitor placed in the mouth would be effective would depend entirely on whether or not it reaches all the susceptible areas of the mouth in sufficient concentration to inhibit acid forma-

tion. Fortunately, this concentration is usually very small. It is not necessary to stop acid formation in most mouths, as most areas of the mouth are almost in equilibrium, naturally. It is only necessary to slow the rate of acid formation to the point that the natural neutralizing influences in the mouth become effective. In 1942 it was found that 2-methyl-1, 4-naphthoquinone was a very effective enzyme inhibitor in concentrations of one mg. per 100 c.c. of saliva. The problem of placing the enzyme inhibitor in the mouth at the proper time was of major importance. This could be done by means of a mouthwash, a dentifrice, or by some other vehicle such as chewing gum. Insofar as it is necessary to have it present a short interval of time after each meal or ingestion of food during which time the maximum acidity would normally be produced, it was decided that chewing gum would be the natural vehicle insofar as it is much easier to carry around and use than either a dentifrice or a mouthwash. In addition, it was decided that insofar as chewing gum contained 60 per cent sugar, there would always be an adequate supply of substrate for the enzyme system to produce acids, provided acids were capable of being produced.

On this basis an experiment was done to determine the effect of vitamin K on caries activity when used in a chewing gum.[14] Each individual was instructed to chew one pellet of gum immediately after each meal or ingestion of food. Before and after the experiment highly accurate mouth examinations were made and chemical and bacteriological tests for susceptibility were performed. There was a very accurate selection of patients. Each test patient presented a minimum of twelve caries-free interproximal spaces and had a plus three or four susceptibility, according to both the bacteriological tests and the chemical test. These individuals would be expected to produce a maximum of carious lesions so that any differences could be judged as significant differences. The control group consisted of either immune persons, those who did not have a sufficient number of caries-free interproximal spaces, or those who did not wish to participate in the experiment. It was found that the experimental group produced 60 to 90 per cent fewer carious lesions than did similar control patients. The control group that consumed chewing gum produced approximately one-half this amount of caries. Unfortunately, the control chewing gum contained calcium carbonate, which from a theoretical point of view should cause a neutralization of acids as fast as they are formed.

On the basis of the foregoing a theoretical explanation of why enzyme inhibitors should inhibit caries has been described. An enzyme inhibitor that produces no other complications has been tested and found to substantiate the theory. On this basis it must be assumed that any enzyme inhibitor that acts on the anaerobic carbohydrate degradation scheme should work equally as well in some type of chewing gum on caries activity.

From a theoretical point of view, normal prewar chewing gum contained 60 per cent sugar, and hence furnished an excellent substrate for the action of the enzyme system. If no other complications were present, it could be assumed that chewing gum should increase the caries activity of the individual. However, the rate of acid production is not the only factor involved. The chewing of gum will produce in most cases a copious supply of saliva and the act of

chewing will force this saliva into all the occluded spaces of the mouth. Thus, if the chewing of gum were uncomplicated by the presence of sugar, the act of chewing should inhibit caries by increasing the rate of neutralization of acids. In view of this, it is clear that chewing gum simply accelerates the rate of acid production and the rate of neutralization. If the final result shows that acid is formed more rapidly, and not neutralized more rapidly than without the chewing gum, more caries should be expected. If the reverse is true, less caries should be expected, and if there is no appreciable change from the normal, chewing gum should have no effect. From actual measurement of acid production in the mouth, chewing gum has less effect than sugars alone, but no controlled clinical experiments have been done on prewar gum. When it was found that calcium carbonate in chewing gum actually decreased the caries activity of a group of individuals, most gum manufacturers placed calcium carbonate in their respective products. Thus, approximately 98 per cent of all the gum on the market today contains calcium carbonate and should be harmless and actually beneficial to most individuals. However, it is the opinion of the author, based upon some cursory experiments, that even with the calcium carbonate present, certain cases of rampant caries are in no way benefited, and may be harmed, by the chewing of gum, particularly those cases in which no stimulation of saliva is possible. This condition may be brought about by an atrophy of the salivary glands or by constant stimulation, such as is observed with those individuals who are classified as "chain chewers."

SUMMARY

On the basis of theoretical considerations, prewar gum should increase caries activity in some individuals, have no effect on others, and be beneficial to some, if not used in excess. Postwar gum should be beneficial to many but under certain conditions may be harmful. In any case, gum would be less harmful than sugar in other forms.

On the basis of theoretical considerations and actual experiments, vitamin K shows promise of controlling caries. More evidence is needed before either gum or vitamin K should be recommended to the general public as a control measure.

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DISCUSSION

DR. EASLICK.—Thanks very much, Dr. Fosdick. Dr. Fosdick's presentation is open for discussion, and the hand up here is Dr. Armstrong's.

DR. WALLACE D. ARMSTRONG.—Well, I am sure Dr. Fosdick knows what I am going to say. I am rising on a point of semantics, and in no way to offer criticism or objection to the rationale in the use of enzyme inhibitor of the nature of a quinone. Vitamin K, I think, is a word which should be restricted to those substances which promote the formation of prothrombin, and should also be restricted to those substances in their natural occurrences. There are two vitamin K's, vitamin K_1 and K_2, one produced in plants and one by bacteria. Now, it happens that there are substances which show vitamin K activity. Actually 2-methyl-1, 4-naphthoquinone on a molecular basis has a higher vitamin K activity than do the naturally occurring substances. These properties which Fosdick and his co-workers discovered, which show that 2-methyl-1, 4-naphthoquinone is an anti-bacteria substance, insofar as it interferes with the production of acids in saliva, has a very real effect but it is an effect which is in no way related to the vitamin K activity of 2-methyl-1, 4-naphthoquinone. As a matter of fact, there is another quinone which has even greater anti-bacteria effect than 2-methyl-1, 4-naphthoquinone, or at least as good, and that is 2,6-dimethyl-benzoquinone, which has no vitamin K activity at all. I suggest that it would be better for the public interest, which is so great in regard to vitamins, since this has not a vitamin effect at all that simply the word "quinone" be used in this description.

DR. FOSDICK.—Dr. Armstrong, I heartily agree with everything you say. Perhaps if I explain why we investigated the problem it would explain how we happened to call the quinone "vitamin K." We had what I thought was an excellent idea. Insofar as most bacteria stop the carbohydrate degradation cycle with lactic acid, or something similar, and do not go through the dicarboxylic acid cycle, we thought it would be well to boost the lactic acid over the hump, and thus cause the lactic acid to be converted to carbon dioxide and water. There was an excellent theoretical scheme, but it didn't work. We tried several things like thiamine and niacin, and various other vitamins to see if we couldn't cause the reaction to proceed, but most of the vitamins, such as thiamine and niacin, seemed to stimulate lactic acid production. This is not surprising now that we know more about it. The other vitamins did not have much effect on the reaction except perhaps the one known as Cerophyl, a mixture of various vitamins. In order to save our work we simply tried all the vitamins and published a paper on the effect of vitamins on acid formation. We studied all the vitamins, and among them was vitamin K. At that time menadione was called vitamin K. Without knowing the exact mechanism we published under the title, "The Effect of 2-methyl-1, 4-naphthoquinone or vitamin K." Insofar as it was in a paper concerning all the vitamins, one naturally associated it with the vitamin, but later on it was definitely found that the action has absolutely no connection with its vitamin activity. Since we published the article perhaps fifty or sixty compounds have been found which have equal activity as far as inhibition of acid formation is concerned. Does that explain the situation?

DR. ARMSTRONG.—Yes, sir.

DR. EASLICK.—Having heard Dr. Armstrong's discussion and Dr. Fosdick's reply, I hope that you health educators will refer to quinones in the future, and not vitamin K.

DR. DONALD A. WALLACE.—Just another point on nomenclature. Wouldn't a better name for this substance be menadione sodium bisulfite? Isn't that what you used?

DR. FOSDICK.—Yes, but it wasn't accepted at the time under that name.

DR. WALLACE.—No, I understand, and I wonder if you would care to comment on the possible effect of the bisulfite portion of the molecule in inhibiting acid formation?

DR. FOSDICK.—I didn't quite understand your question.

DR. WALLACE.—I don't recall the reference, but sometime between the time of the paper on this chewing gum was published and now, there was a report in the literature* to the effect that sodium bisulfite would inhibit acid formation also. It was used, I think, in considerably higher concentration than in the chewing gum.

DR. FOSDICK.—We tested the effect of sodium bisulfite as an acid inhibitor and found it was absolutely uneffective in concentrations that could be used practically.

TUESDAY, SEPTEMBER 9

Afternoon Session

(Kellogg Institute Auditorium)

DR. EASLICK.—This afternoon's activity is 100 per cent audience participation because the evaluating committees are about to swing into action. I have called you together for a few minutes as a group here in the Auditorium of the Kellogg Institute in order to present a few instructions which the Planning Committee for this Conference thought would be helpful.

I regret that not all of you have been assigned to the evaluating group of your first choice. Such an assignment was impossible because two of the committees were just too popular. I hope that you will accept your assignments graciously and work just as hard as if they were your initial selections. If any of you have failed to note down your committee, you may check your assignment at the table in the upper lobby.

GROUP LEADERS

In order that work might start faster, group leaders have been selected in advance. I appreciate most sincerely their willingness to serve. One of the early pieces of business will be the selection of a secretary.

I am going to read the names of the leaders and ask each one to rise as his name is read so that everyone can get a good look at his group's Simon Legree:

Committee I (The Mechanism of the Caries Process)—Dr. George W. Teuscher, Professor of Pedodontics, Northwestern University, Chicago.

Committee II (Relationship of Systemic Disease to Dental Caries)—Dr. Ruth Martin, Professor of Dental Pediatrics, Washington University, St. Louis.

Committee III (Effectiveness of Tooth Brushing, Gum Chewing, Dentifrices, and Mouthwashes in Control of Caries)—Dr. Floyd D. Ostrander, Associate Professor of Dentistry (in Therapeutics), University of Michigan.

Committee IV (Utilization of Fluorides and Silver Salts in Control of Caries)—Dr. Albert L. Russell, Director, Division of Dental Health, State Department of Health, Pierre, South Dakota.

Committee V (Relationship of Vitamin and Mineral Ingestion to Control of Caries)—Dr. Philip Blackerby, Jr., Director, Dental Division, W. K. Kellogg Foundation, Battle Creek, Mich.

*Dreizen, S., Mann, A. W., Spies, T. D., Carson, B. C., and Cline, J. K., *J. D. Res.* 26: 93, 1947.

Committee VI (Contribution of the Type of Diet to Caries Control)—Dr. J. M. Wisan, Chief, Division of Dental Health, State Department of Health, Trenton, N. J.

CONSULTANTS

At this time I should like to announce also the consultants for each group. As you will note, they come largely from our guest faculty.

I	II	III	IV	V	VI
Chase	Mrs. Harter	Hall	Arnold	Armstrong	Becks
Miss Crowley	Kerr	Hill	Bibby	Beeuwkes	Jay
Fosdick	Robinson	Hine	Knutson		Miss Johnson
Stephan		Kesel	Zander		Stearns
Miss Tramp		Wallace			
Williams					

These people are assigned as resource people to help you in obtaining information, in raising pertinent questions, or in checking the reliability of information.

GROUP ACTIVITY

As I stated yesterday, and as I feel, most sincerely, the success or failure of this Conference will depend on your activity in the various groups. I should like to call a few signals, therefore, which the Planning Committee thought would help make these afternoon sessions productive.

In addition to the selection of a chairman by our Committee and the selection of a group secretary by you, some other aids have been arranged. As you now know, a series of questions will be provided for each one of you which, it would seem, should be answered before the writing of conclusions can be attempted. Each of you also will be given copies of the references used by the speakers in your particular areas.

The initial activity for each group, after locating its meeting room, will be organization. Your chairman will call the roll, see that everyone is thoroughly introduced and properly "tagged," and distribute the materials with which you will work. Probably the next bit of business will be the selection of a secretary. Then, according to the program, you will go over the questions in your specific area, discuss them thoroughly, and finally, note down any additional questions which you agree may have to be answered in order to write conclusions. At the conclusion of this discussion your leader will assign each one of you a group of references to examine critically and appraise carefully to see if the conclusions are warranted by the evidence. At the discretion of the chairman he then will alternate discussion and study periods throughout the balance of the week until the most scientific answer that the available data permit has been found for each question. By Saturday morning the answers to the various questions will be converted into brief conclusions that will be presented to the entire group for discussion, acceptance, or adoption. You will be expected to defend your conclusions.

When Dr. Hall is badly needed by your group or you wish to use some other group's consultant or you wish to see me for more precise directions, a message should be sent to the table in the lobby. Someone will serve constantly each afternoon as a messenger. When you need references you should go directly to the Library and sign out with an assistant the journal or volume which you require. Many other references on dental caries will be found available there.

Here my instructions end. Will each chairman come to me and get the materials for his group? Will each consultant and student go directly to his study room?

2:00 (Seminar Rooms, Kellogg Institute)
5:00 Organization of Individual Committees and Determination of Questions That Have to Be Answered in Order to Evaluate the Present Status of the Caries Control Measures Assigned to Each Committee.

(Meeting adjourned until 9:00 a.m., September 10, 1947.)

WEDNESDAY, SEPTEMBER 10

Morning Session

(Auditorium, School of Public Health)

DR. EASLICK.—Now that the evaluating committees have been organized and have begun to function I anticipate that there may be a more lively discussion from the floor; so it may be a bit more difficult for the speakers hereafter. If I remember the program correctly, twenty-minute discussion periods are scheduled this morning following each lecture. And I anticipate that they will be used.

This morning we are scheduled to proceed further with the evaluation of caries-control technics. The first speaker comes from the School of Dentistry of the University of Illinois in Chicago where he serves as Professor of Applied Materia Medica and Therapeutics. He is Dr. Robert G. Kesel, and I can promise you that during the next forty-five minutes you will hear a very fine discussion of "The Effectiveness of Dentifrices, Mouthwashes and Ammonia-Urea Compounds in the Control of Dental Caries." And I say that I can promise you because I also heard him talk this past June at the University of Northwestern Conference for Children's Dentists, and he did a beautiful job. It is a pleasure to have you repay my visit to Chicago, Dr. Kesel.

THE EFFECTIVENESS OF DENTIFRICES, MOUTHWASHES, AND AMMONIA-UREA COMPOUNDS IN THE CONTROL OF DENTAL CARIES

ROBERT G. KESEL, SCHOOL OF DENTISTRY, UNIVERSITY OF ILLINOIS, CHICAGO, ILL.

Until recently I have been skeptical of the possibility of a dentifrice or a mouthrinse influencing significantly, for any prolonged period of time, the oral bacterial flora, and skeptical also of its ability to reduce caries activity in any significant amount. There are a number of factors that operate against the effective use of such preparations; for instance, salivary dilution. When a dentifrice or mouthrinse is taken into the mouth its effectiveness would seem to be only momentary because of the rapid dilution from saliva, particularly when most of these agents contain flavoring oils which tend to stimulate salivary flow. Then there is another limiting factor: that of penetration. Can the active ingredient in the dentifrice or the mouthrinse reach the vulnerable areas on the surface of the teeth where decay is actually occurring?

Muntz and Miller[1] have reported on the impermeability of plaque material. By a clever device, they homogenized bacterial plaques that had been scraped off teeth. They put this homogenized suspension in fine capillary tubes, and by centrifuging the material they were able to lay down plaques of varying thickness in the bottom of these tubes. Then they exposed these plaques to various anti-carious agents to determine what the permeability of the plaque material would be, and they found evidence that even such diffusible things as urea and glucose did not readily penetrate this artificially precipitated plaque material.

Another factor that operates against the effective use of a dentifrice or mouthrinse is the fact that many of the active ingredients employed are inactivated or are rendered less effective by organic material, and we know that there is abundant organic material, microscopically speaking, and that the ingredients must penetrate if they are going to be effective.

Still another limiting factor, and a very real one, I think, is the necessity for patient cooperation. Not many people brush their teeth. However, if we could produce satisfactory and conclusive evidence that tooth brushing or mouth rinsing is effective in reducing caries activity, I think that a much larger number of people could be stimulated into using the toothbrush or a mouthrinse. Before this state can be obtained more conclusive evidence must be secured.

A review of the literature regarding the effectiveness of a medicated mouthrinse reveals that a study was made a few years ago at Johns Hopkins University[2] in which mouths were rinsed with 1,000 c.c. of water. The 1,000 c.c. were divided into 25 c.c. portions, and these were used consecutively for forty rinsings. Bacterial counts were made of the first, the fourth, the twentieth, and the fortieth rinsing. The number of bacteria that were removed from the mouth by tap

water was found to be reduced in the consecutive rinsings. There was an average colony count of 2,700,000 in the first rinsing, and in the fortieth rinsing there were still over a million bacteria present. While there was a reduction, it seems that the supply of bacteria in the oral cavity is almost inexhaustible.

The experimenters observed that during the day there is a marked increase in the number of bacteria in the mouth if the individual fasts. They found that if a subject rinsed his mouth at 9:00 o'clock in the morning and a bacterial count was made of that rinsing, and then if counts were made of hourly rinsings throughout the day, at the end of seven hours there was an increase of about 300 per cent in the number of bacteria that could be removed from the mouth provided that no food was eaten or the teeth brushed. They admit that these counts are not necessarily an indication of what is left in the mouth, but they feel that the results are acceptable evidence of tremendous increase in the number of bacteria that can be removed, and that they are indicative of an increase in the number of bacteria left in the oral cavity.

They termed this increase the diurnal tide of bacteria, and they used it to test the effectiveness of various mouthrinses and dentifrices. The control rinsing was taken at 9:00 A.M. and immediately afterward the individual would rinse his mouth with the test material. Rinsings were collected for culture at hourly intervals throughout the day to determine the increase in the bacterial population. The results obtained with various agents were compared. Only one of the germicidal agents used (hexylresorcinol) prevented the diurnal tide. I don't believe this study has ever been confirmed; in fact, other experiments have been performed that raise doubt about the effectiveness of hexylresorcinol in controlling the oral bacterial population for any long period of time.

Another study concerned with the use of a medicated rinse was reported in 1934 by Grove and Grove.[3] They claimed that rinsing the mouth with a very weak ammonium hydroxide solution had a cleansing effect on the teeth and also a reducing influence on caries activity.

More recently Hanke and his co-workers[4] in Chicago have claimed effectiveness for an organic mercurial solution known as Solution 58. They tested a number of salts of heavy metals to determine what their effect might be, particularly on what they called the plaque-forming organisms. A fungicide was sought which would kill the filamentous bacteria that constitute the basis for the plaque, that would lift these plaques from the tooth surface, or at least reduce the plaque thickness. The study reports that mouths treated with Solution 58 showed decided improvement in cleanliness, and also that the solution markedly reduced fermentation. I don't believe that there has been confirmation of their findings as yet. The question of possible toxicity of the mercury in the manner used has not been answered to the satisfaction of all.

A few years ago there was an experiment conducted by Wach, O'Donnell, and Hine,[5] in which they used urea and quinine to determine what the effect might be on the oral flora and the rate of fermentation by saliva. The rationale behind the combination of these materials is that quinine is not only an antibacterial agent, but also an antienzyme. It is sometimes used in hot climates

to prevent the souring of milk. Quinine operates more effectively in an alkaline environment. The urea was included primarily to provide alkalinity so that the quinine would have its optimal antibacterial and antienzymatic effect. A group of dental students used this mouthrinse for fourteen days, and an appreciable reduction in the number of aciduric bacteria in their oral cavities was obtained. However, no way was found to mask the bitter taste of the quinine, and so I doubt very much that the practical use of a solution with quinine in it could be widely effective in caries control because of this lack of palatability.

Coming to our own recent studies,[6] we have been particularly interested in those individuals who have no dental caries and no lactobacilli in their saliva, and yet consume the usual amount of carbohydrate foodstuffs in their diets. We wondered why those individuals could be that way. In reviewing the literature, we found an experiment that Bunting[7] had conducted a number of years ago. He had caries-immune individuals rinse their mouths with acidophilous milk, and he found a rapid disappearance of lactobacilli from their oral environment. We had a few dental students associated with us who could be classed as so-called caries-immune. We had them rinse their mouths with acidophilous milk that we had prepared by inoculating sterilized milk with an oral strain of lactobacilli and incubating it for twenty-four hours. The mouths of the caries-resistant individuals were rinsed with this milk containing heavy suspensions of lactobacilli. Saliva cultures were made at hourly intervals to see what would happen to these organisms, and it was found that they began to disappear almost immediately. At the end of nine to twelve hours they would be eliminated from the oral cavities of these individuals.

We were aware also of the work of Clough[8] in which he cut wells in agar plates, the agar having been previously seeded with lactobacilli. Caries-immune saliva was placed in these holes and upon incubation a zone of inhibition was found around these wells. This result demonstrated that there is some antibacterial factor for lactobacilli in caries-resistant saliva. Clough found that this material was in the supernatant portion of centrifuged saliva. It was not in the sediment.

Hill[9] reported in 1939 that when caries-immune saliva was inoculated with lactobacilli and incubated for twenty-four hours, these organisms were destroyed despite the addition of glucose to the caries-immune saliva. We repeated his work and obtained the same result. We found, however, as he did, that if too many lactobacilli were added to the caries-resistant saliva the inhibiting factor was overcome. We discovered more or less by accident that if this caries-resistant saliva were allowed to stagnate in the incubator for a few days the inhibiting factor would be considerably increased. We learned this by incubating saliva at body temperature for varying periods up to two weeks. At the end of the selected incubation period the stagnated saliva was passed through a Seitz filter to obtain a clear, colorless filtrate. To that filtrate we added saliva that had been freshly collected from a caries-active individual, saliva that we knew contained large numbers of lactobacilli. Glucose was added to the mixture to enhance the growth of these organisms. One-tenth of a cubic centimeter of the

combination was spread over the surface of a tomato agar plate to provide a record of the number of lactobacilli present in that mixture at the beginning. Then the mixture was incubated for seventy-two hours. At the end of twenty-four, forty-eight, and seventy-two hours' incubation, subcultures were again made on tomato agar.

The lactobacilli that were in the caries-active saliva disappeared after twenty-four hours' contact with saliva (collected from a caries-immune individual) which had stagnated for one week. We began to suspect that whatever the inhibiting factor, it was the result of bacterial activity because we found that the inhibiting ability increased steadily up to eight days during an incubation period. Beyond eight days there seemed to be no significant increase. If this inhibition was due to bacterial activity, then by placing immune saliva in culture medium to stimulate bacterial activity we should be able to increase the inhibiting factor. And we did. We inoculated immune saliva into beef broth, incubated it for a week's time, and then checked the filtrates of such cultures against caries-active salivas. We found that such treatment definitely increased the ability of the culture filtrates to destroy lactobacilli. We wondered what species of bacteria were growing in cultures of saliva that produced an inhibiting action on lactobacilli. We decided to subculture the cultures of immune saliva at daily intervals up to one week to see which species occurred consistently and if in pure culture they elaborated an inhibiting factor. One hundred five different bacterial species grew with sufficient consistency in cultures of immune saliva to lead us to believe that they might be responsible for the inhibitory action. These types were grown in pure cultures, passed through filters, and their filtrates tested against lactobacilli. As long as lactobacilli were the organisms in whose inhibition we were particularly interested, we decided to use pure cultures of them in the inhibitory tests rather than use the varying numbers of lactobacilli that occur in freshly collected samples of caries-active saliva. We obtained from the American Type Culture Collection at Georgetown University the Hadley strain of lactobacilli. A twenty-four-hour culture of it was placed in the culture filtrate prepared from immune saliva so that it would be in a dilution of one part lactobacillus culture to 100 parts of filtrate. We arbitrarily set up the standard that if the filtrate contained a strong amount of the inhibiting principle it would destroy lactobacilli during twenty-four hours' contact. If the inhibiting substance was only weakly present it would permit some growth of lactobacilli after twenty-four hours' contact, but would have destroyed all the lactobacilli after seventy-two hours' contact. Filtrates that did not contain the inhibiting substance would permit the abundant growth of lactobacilli during seventy-two hours' contact. This was the standard for our test of the inhibiting potency of the filtrates obtained from these 105 different organisms. Only one of the 105 caused the inhibition of lactobacilli; that was *Bacterium aerogenes,* one of the coliform types of bacteria. We grew this organism in combination with other organisms we had obtained from caries-immune saliva to determine if it might have a symbiotic-synergistic effect. We found a gram-positive, chain-forming bacillus (that we have not been able to identify)

which would not inhibit lactobacilli by itself, but which in combination with *B. aerogenes* definitely increased the ability of *B. aerogenes* to destroy lactobacilli.

Then we performed a series of experiments to see whether or not this inhibitory factor might be antibiotic like penicillin. If it were, it should be effective in high dilutions because, as you know, penicillin can be diluted many times and still be effective. We found that if a filtrate that was definitely inhibitory was diluted with an equal volume of beef broth it began to lose its inhibiting power. If it was diluted with two volumes of broth the inhibition was practically gone, and if diluted with three, the organisms grew as well as they did in the control of glucose beef broth.

Faced with these results, we decided to determine if we could increase the inhibiting potency by concentrating these filtrates through evaporation. The evaporations were done, some in vacuum at room temperature and some in a hot oven. We concentrated these inhibiting filtrates, some to 50 per cent of their original volume, some to 75 per cent, and some to dryness, and then resuspended the residue in beef broth at 40 per cent of the original volume. We found to our surprise that the inhibiting factor was lost in direct proportion to the amount of evaporation. Thus the filtrates evaporated to dryness completely lost their inhibiting properties. It became clear that the inhibiting substance was volatile and was being removed in the distillate.

We began to analyze the distillate and we soon found that always in those filtrates that had the inhibiting factor, ammonia nitrogen was detectable, and that the amount of ammonia nitrogen was proportionate to the degree of inhibition that filtrate would exert on lactobacilli.

Next, to check on the possible importance of ammonia we added various ammonium salts directly to the culture medium and we found that these salts would do the same thing as the ammonia produced naturally in the culture of saliva. However, we found that acid salts of ammonia would not inhibit. This observation was similar to one made by Grove and Grove[3] a number of years ago. They had observed that the basic salts of ammonia seemed to have a beneficial effect on the oral cavity, whereas the acid salts did not. We found that ammonium carbonate was the most effective of all of the ammonium salts in inhibiting lactobacilli.

The observation about the alkaline salts raised the question of whether the ammonia was the inhibiting factor or whether it was the alkalinity that the ammonium salt gave to the medium. To answer this question, we prepared two solutions, each one of them having the same hydrogen-ion concentration (one of ammonium acetate and one of sodium acetate). We added lactobacilli in varying dilutions to these two types of alkaline solutions, and we found that the ammonium salts would definitely inhibit the growth of the organisms, whereas two and three times the concentration of sodium acetate had no effect whatsoever.

With this information we then proceeded to a clinical study of the possible effectiveness of ammonia in a dentifrice and of a mouthrinse on the oral bacterial population. We experimented with a number of different ammonium salts. We wanted to use ammonium carbonate because in vitro we knew that it was the most effective, but we didn't think it was satisfactory for a dentifrice because it is unstable, it has an ammoniacal odor and taste, and produces slight burning when it is used in the concentration we desired. We decided upon dibasic ammonium phosphate because it was shown to have an inhibiting effect and it doesn't have the marked ammoniacal taste and odor that the ammonium carbonate has.

We knew of the work that Howe[10] did several years ago when he showed that low concentrations of phosphates, including ammonium phosphate, had a stimulating effect on bacterial growth, including lactobacilli. Therefore, we had to use diammonium phosphate in sufficient concentration to be inhibitory and not stimulating. That is why we set up as the active ingredient in our first formula, 5 per cent dibasic ammonium phosphate.

A group of fifty-five individuals who had very active dental caries was selected. They had had, with a few exceptions, at least ten cavities filled in the two years prior to the study and had lactobacilli counts of 50,000 or more per cubic centimeter of saliva on four successive days prior to the use of the dentifrice. Ten of these fifty-five were used as controls; they used a dentifrice that had the identical composition of the experimental dentifrice except that the dibasic ammonia was omitted. The lactobacilli counts for the control group before and after five months' use of the dentifrice was given in Table I.

TABLE I

CONTROL GROUP: EFFECTS ON LACTOBACILLI COUNTS OF FIVE MONTHS' CLINICAL USAGE OF MOUTHRINSE AND DENTIFRICE CONTAINING NO AMMONIA

SUBJECT	A	B
1	249,000	234,000
2	810,000	870,000
3	200,000	155,000
4	73,000	121,500
5	110,000	128,500
6	485,000	365,000
7	200,000	412,000
8	550,000	330 000
9	400,000	415,000
10	295,000	325,000

There was no appreciable, no significant change in the number of lactobacilli.

Table II shows the lactobacilli counts for the forty-five who used the dibasic ammonium phosphate. Column A gives the counts at the beginning of the experiment, Column B the counts after five months, and Column C the counts at the end of one year. During the year we lost several of these subjects, so that we completed the year with thirty-three of the original group of forty-five. Thirteen had their lactobacilli count reduced to zero. The others continued to show lactobacilli surviving although at reduced levels.

TABLE II

TEST GROUP: EFFECTS ON LACTOBACILLI COUNTS OF FIVE MONTHS' CLINICAL USAGE OF A MOUTHRINSE AND DENTIFRICE CONTAINING AMMONIA

SUBJECT	A	B	C
11	698,000	40,000	No growth
12	450,000	523,000	
13	202,000	75,000	
14	410,000	81,000	1,500
15	561,000	No growth	2,500
16	509,000	74,000	12,500
17	1,400,000	58,500	
18	148,000	42,000	
19	1,500,000	19,000	110,000
20	338,000	140,000	
21	562,000	110,000	18,000
22	150,000	10,000	
23	51,000	1,200	No growth
24	269,000	16,500	No growth
25	675,000	No growth	3,000
26	375,000	42,000	
27	260,000	30,000	
28	1,450,000	160,000	30,000
29	610,000	33,500	No growth
30	300,000	1,000	5,000
31	165,000	40,000	
32	470,000	30,000	No growth
33	900,000	145,000	15,500
34	540,500	70,000	No growth
35	1,250,000	16,000	No growth
36	1,500,000	320,000	40,000
37	370,000	110,000	12,000
38	260,000	No growth	
39	145,000	25,000	No growth
40	495,000	160,000	6,000
41	525,000	10,000	No growth
42	265,000	No growth	15,000
43	73,000	No growth	
44	110,000	35,500	1,500
45	280,000	6,500	No growth
46	1,100,000	110,000	40,000
47	750,500	210,000	30,000
48	820,000	2,500	No growth
49	900,000	160,000	110,000
50	585,000	No growth	2,500
51	294,000	70,000	18,000
52	160,500	250	No growth
53	485,000	No growth	
54	315,000	90,000	No growth
55	755,000	20,500	2,000

About this time Fosdick raised a question. We had reported that there was no change in the hydrogen-ion concentration in the salivas of carious patients under the dentifrice treatment when glucose was added to them. Fosdick asked if the lactic acid being formed by these salivas was being buffered by the ammonia that was being introduced. We checked on that question and found that there was definitely some glucose conversion, but that the resultant acid was being buffered.

We then sought some agent to combine with dibasic ammonium phosphate which would prevent this lactic acid formation, and which might also have a greater inhibiting effect on lactobacilli. We immediately thought of urea because of Stephan's work[11] and because of our experiments with quinine and urea

in 1941. We tried various combinations of urea with dibasic ammonium phosphate. We wanted to use a small concentration of urea because we believe that large concentrations may be detrimental to the deaminating enzyme systems as well as to the fermentative types. At first a concentration of 10 per cent urea and 5 per cent dibasic ammonium phosphate was tried in in vitro tests. We found that such a combination would definitely prevent lactic acid formation. We proceeded to reduce the amount of urea and found that we could take it down to 3 per cent and it still would prevent lactic acid formation by caries-active saliva when glucose was added.

We learned that a combination of 3 per cent urea and 5 per cent dibasic ammonium phosphate will do what neither one will do alone in regard to the hydrogen-ion concentration, the total titratable acidity, the lactic acid formation, the ammonia nitrogen production, and the aciduric flora in caries-active salivas. Table III gives a comparative summary of this study which was done on thirty-five salivas. The 3 per cent urea solution permitted some acid formation after four hours' contact in the incubator, but after twenty-four hours and forty-eight hours the pH was well up on the alkaline side. The amount of lactic acid formed was stimulated by the 3 per cent solution. About three and one-half times as much lactic acid was formed in the solution with 3 per cent urea as developed in the saliva-glucose mixture without the urea, which was similar to Stephan's observations. The ammonia nitrogen level increased during the forty-eight-hour period, and the 3 per cent urea was effective in eliminating lactobacilli after forty-eight hours' contact.

The diammonium phosphate showed a somewhat different result. The pH was on the alkaline side at the beginning, but there was a reduction toward the acid side after forty-eight hours' contact. The same was true of the total titratable acids. However, there was not nearly as much lactic acid formed as there was with the urea mixture. There was about as much lactic acid with 5 per cent dibasic ammonium phosphate as there was in the saliva-glucose mixture without the diammonium phosphate. There was the same inhibition, however, of the acid-producing bacteria.

When these two solutions were combined they maintained a high pH, no titratable acids were present during the forty-eight hours' incubation, and there

TABLE III

	3% UREA SOLUTION			5% DIAMMONIUM PHOSPHATE SOLUTION			COMBINED SOLUTION		
	4 HRS.	24 HRS.	48 HRS.	4 HRS.	24 HRS.	48 HRS.	4 HRS.	24 HRS.	48 HRS.
pH	6.0	7.6	7.8	7.8	7.4	7.0	7.8	7.8	7.8
T.T.A.	0.8	Alk.	Alk.	Alk.	1.0	2.8	Alk.	Alk.	Alk.
Lactic Acid	2.283	5.523	7.821	0.18	1.338	1.690	0.202	0.16	0.21
Ammonia Nitrogen	0.04	0.24	0.31	3.361	2.74	2.182	3.66	3.64	3.43
Aciduric Bacteria	190,000	45,500	0	270,000	L.A. 0 C -- 11,200	0	295,000	0	0

was a lack of lactic acid formation. Whereas urea had stimulated lactic acid formation and dibasic ammonium phosphate had not inhibited it, there was a synergistic effect between the two that prevented lactic acid formation. A high

ammonia nitrogen level was maintained and, as we would expect, no lactobacilli grew after twenty-four hours' contact. Therefore, we modified our formula to include as the active ingredients, 5 per cent dibasic ammonium phosphate and 3 per cent urea.

We studied our original group of thirty-three individuals using the ammonia-urea preparation for another year. Table IV shows the number of lesions that formed during the two-year brushing period. I hesitate to show these results, for I realize the group is not nearly large enough to be statistically significant and not adequately controlled because these individuals had been very caries active before the brushing treatment. They had had many of the vulner-

TABLE IV

NUMBER	AGE	LESIONS BEFORE R	CAVITIES 2 YEARS AFTER R	LAST COUNT
11	14	20	1	4,500
14	17	18	2	None
15	16	24	1	None
16	15	26	2	10,000
19	26	18	0	None
23	31	1	0	None
24	29	14	0	1,200
25	26	12	0	6,500
28	11	19	1	8,500
29	14	20	1	6,000
30	12	17	0	None
32	22	12	0	1,500
33	12	14	0	10,000
34	8	12	1	2,500
35	7	8	0	None
36	32	18	3	16,000
37	11	14	0	None
39	17	18	0	None
40	15	20	2	20,000
41	13	17	0	None
42	12	23	1	None
44	12	22	0	None
45	23	16	1	None
46	21	22	0	11,000
47	33	19	0	16,000
48	34	14	0	1,500
49P	25	28	2	30,000
50	11	12	0	None
51	19	11	0	None
52	23	12	0	None
53	23	14	1	None
54	5	18	1	None
55	24	16	0	None

able surfaces already restored by good operative procedures; therefore, they did not have as many susceptible areas when they went on the study. Then, too, there is the element of conscientious tooth-brushing. We had an interested group to work with, and they did a good job, we believe, of using the dentifrice and the rinse at least twice a day. We had no adequate control. We don't know what would have happened if these individuals had conscientiously used an ordinary dentifrice or just the toothbrush during this period of time. However, I shall show these results because this is a discussion conference, and I shall report to you what we have seen in this group over a two-year period.

Nineteen of the thirty-three individuals reported in Table IV had no new lesions develop during the two-year experimental period. The fourteen that developed caries had twenty new lesions among them. However, we can't place much weight on these clinical data. The study must be done on a large enough group to be statistically significant and it must be more adequately controlled. This fall we hope to institute an oral hygiene study on about 2,000 school children to determine the effectiveness of the toothbrush and dentifrices of various compositions, including our formula, in preventing or reducing caries activity.

The question has been raised about the possibility of influencing dental caries activity by the use of some agent that would reduce the plaque material on the tooth surface. We knew of Grove and Grove's observation[3] of the effect of an ammonia mouthrinse on plaque formation, and so we decided to check the effect of the ammonia-urea solution. Teeth were stained with brilliant green and then the mouth was rinsed with plain water for two minutes. Stain was left on the teeth although water was effective in removing some of it. Staining indicated that much plaque material had been removed after a two-minute rinsing wih the ammonia-urea solution. We felt perhaps there was a decoloring effect of these drugs on brilliant green, and we checked on that possibility. There seems to be no decolorizing effect of the ammonia-urea solution when mixed with the brilliant green in large or small amounts in vitro. We believe, therefore, that the loss of color on the teeth is due to a change in the plaque content or the amount of plaque on the tooth surface, and not to any reducing effect on the stain by the mouthrinse. It may be that there are a number of other solutions that will produce identical or even better results. That is a problem that requires further investigation.

The manner in which these ammonia-liberating agents work is still a mystery to us. Nitrogen is a requirement for bacterial metabolism and when we put ammonia in the oral cavity it would seem that we are providing nitrogen, and therefore, we should get a stimulation of bacterial activity rather than a reduction. But that does not occur, at least not for the acidogenic flora. We feel that probably on the surface of the tooth in this bacterial plaque there is a continuous fight for survival by two antagonistic types of bacteria, and that it may be possible, by the application of ammonium salts, to influence this bacterial population so that we get a rearrangement with the proteolytic types in a dominant position. The ammonifying organisms are present in almost all mouths, even in caries-active plaques, but apparently they are suppressed by the presence of the acid-producing organisms and by the fact that sugar will prevent the deamination process.

That is why I am changing my skepticism expressed at the beginning of this discussion in regard to the possibility of any dentifrice or mouthwash having any prolonged effect on the oral flora. It may be that the use of an agent or agents that will depress only certain types of organisms while stimulating others may have a much longer effect, because of the stimulation provided for the natural antibiotic mechanisms, than one that depresses all bacterial life. In other words, the rearrangement in the bacterial population may continue the effect initiated by the specific antibacterial agent.

We feel that the organic composition of saliva may play a big role in the types of bacteria that are living in the oral cavity. The inorganic constituents of saliva are probably important for the effect they have on the end products of bacterial metabolism, but the organic composition of saliva may have a very significant influence on the types of organisms that are living in the mouth. It may be that age, nutrition, metabolic state, and so on, can influence the organic content of saliva and thereby influence significantly the bacterial population. We believe that this flora is susceptible to very minute changes in the oral environment, perhaps changes that are too insignificant to measure by the usual chemical analyses. If salivas are subjected to the microbiologic assay technic of analysis which we are now doing in analyzing for the amino acid content of saliva, it may be that we shall be able to determine why some individuals have in their oral cavities one type of flora, whereas others do not. I am getting well into the realm of conjecture, however, and I think it is time to stop and let you ask questions.

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DISCUSSION

DR. EASLICK.—Thank you very much, Dr. Kesel. I regret that our lantern failed to work and I am glad you took it so good-naturedly, and I am glad we got it repaired to find out about that conjectural group down in Illinois. Dr. Kesel's discussion, report, and presentation are open for discussion.

DR. ALLEN O. GRUEBBEL.—I should like to ask Dr. Kesel in what way the oral flora of the mouth was changed after the urea and dibasic ammonium phosphate were used?

DR. KESEL.—By disappearance of the lactobacilli. We haven't noticed any other change. No change has been noted because no quantitative study has been done on the other types of organisms in the plaque. That is something that must be determined.

DR. WALLACE D. ARMSTRONG.—Dr. Kesel knows that Grove and Grove produced evidence that the ammonia content of a group with natural caries immunity was higher than for people who had a large amount of caries. This finding was not confirmed by Bunting and White. I wondered if you made any quantitative analyses for ammonia, or what your findings might be in that regard?

DR. KESEL.—Yes, we have. We checked on that angle and more or less substantiated the findings of Bunting and White and also of Youngberg. We could not recognize any consistent difference in the ammonia content of saliva spit out from the mouth. We feel that it is what happens in the bacterial plaque on the tooth surface that is significant, the ammonia being liberated there in small amounts and over a long period of time, not in amounts sufficient to alter appreciably the ammonia content of the saliva as it is collected and spit from the mouth.

DR. LEONARD S. FOSDICK.—I have about three questions to ask. Have you analyzed the ammonia content of the plaques from immune and susceptible individuals? That is number one. And number two, in the summary chart that you have presented, how long after the brushing of the teeth with your solutions did you gather the saliva for incubation? And number three, were the patients on this test instructed to brush their teeth immediately after eating or at any time that they so wished?

DR. KESEL.—Let's take them in order. Question one, have we analyzed the plaque content directly for its ammonia? We have not, but we realize that it is something that should be done if possible. Question two, was this saliva collected soon after brushing the teeth with the ammonia preparation? The ammonia preparation had not been used on the individuals from whom these salivas were collected. It was collected from new patients that were highly caries active. They had reported to the clinic about ten o'clock in the morning, so that it was several hours after they had brushed their teeth. The third question was, when were the patients instructed to brush their teeth? We began this experiment originally by asking them to brush their teeth night and morning, which would follow the usual hygienic procedure, and many of these individuals have continued to just use these two brushings. There are some of the subjects who were much more resistant to a reduction in their lactobacilli counts and we have had them brush their teeth immediately after meals and again before retiring.

DR. EASLICK.—Dr. Williams, did you have a question?

DR. NED B. WILLIAMS.—Dr. Easlick, I should like to ask Dr. Kesel this question: In the counts for lactobacilli, for which you recorded "no growth," were the dilutions altered at the time that you first noted the counts were lower, so that the record "no growth" indicates failure of growth even from undiluted saliva?

DR. KESEL.—You mean in these clinical specimens?

DR. WILLIAMS.—That is correct.

DR. KESEL.—The specimens are always diluted. We followed the Hadley technic and diluted in Jay's broth and then that dilution, which is a one-in-five dilution, was used for the plating. There was no further dilution than that.

DR. WILLIAMS.—Did you examine the broth by smears when the plate from a saliva-broth dilution was negative?

DR. KESEL.—No.

DR. EASLICK.—Have you finished, Dr. Williams?

DR. WILLIAMS.—I have one more question. In your check-up on dental caries, how did you determine whether there were new lesions that appeared after the treatment had been completed? Was that by both clinical and x-ray examination?

DR. KESEL.—By both clinical and x-ray examination.

DR. DONALD A. WALLACE.—Is it correct that you used the urea-quinine rinse to remove the plaques in those pictures you showed?

DR. KESEL.—In those colored slides that we showed, the rinse was dibasic ammonium phosphate and urea, not urea-quinine. If I said urea-quinine, I am sorry.

DR. EASLICK.—Is there any further discussion of Dr. Kesel's report? Dr. Williams?

DR. WILLIAMS.—If I might just come in here again. I think that the reports on lactobacilli counts that are made in many papers are quite important since we have attached so much importance to them. I feel that although we are following the technics that have been reported from the University of Michigan in doing lactobacilli counts, it is important

to check on all of those counts which according to that technic show no growths because the lactobacilli may very easily be there. Therefore, reports of no growth when a dilution of the saliva is used do not show, directly, what might be in that saliva.

DR. EASLICK.—Dr. Jay?

DR. PHILIP JAY.—That particular dilution was arrived at after considerable experimenting with different dilutions. We have found one-tenth of one c.c. of a one to five dilution most satisfactory.

DR. KESEL.—These salivary counts are all taken on the rising sample of saliva first thing in the morning before any toothbrushing or eating has taken place. I doubt if there would be any continuing effect of the ammonia action over the night and into this morning specimen. If we had positive broths in the tubes and negative plates, we would make smears of the positive tubes, but we did not smear the negative tubes from which the dilution of the tomato agar plates was made.

DR. JAY.—How did you know the tube was positive if you didn't smear it?

DR. KESEL.—By examination.

DR. JAY.—You rarely find a sterile broth culture. There is always some growth in it although it may not be lactobacilli growth.

DR. KESEL.—That is true, but there were no lactobacilli in these broth cultures that had been streaked and plated.

DR. JOHN W. KNUTSON.—I might say it is my impression that too much fuss is being made about the smearing of these negatives. If the standard procedure is being used, the negatives as they come out of that procedure should mean as much as the positives, and undoubtedly do. In other words, you get a count of 300,000; that is perhaps no more accurate than negatives. The important thing is always to use the same procedure on your controls as you do on your experimentals.

DR. EASLICK.—Is there any further discussion?

DR. F. A. ARNOLD.—In regard to the negative plates and the positive broths, I think in our fluoride areas we have been carrying out the technic. Dr. Jay and I have been smearing the broths of any negative plate cultures. Positive broths and negative plates have occurred in only 2 or 3 per cent of the cases.

DR. BASIL G. BIBBY.—Might I suggest the lactobacillus counts might be avoided if you didn't bother to do them?

DR. EASLICK.—Does anybody want to discuss Dr. Bibby's report?

DR. HAMILTON B. G. ROBINSON.—I should like to ask Dr. Bibby if he has another method which we can substitute?

DR. BIBBY.—Yes, do it by examination.

DR. ROBINSON.—Isn't caries too slow a process to follow by clinical examination when we are after results? We have a biological test here. I think there is a tendency to overlook this despite the fact that I am one of the group who feel that the count isn't as accurate as it might be. The salivary lactobacillus count is a biological test that we can use to substitute for, and to augment, clinical studies. I don't think that you should take lactobacillus counts alone. They should be correlated with clinical studies. Caries is such a slow process that we have to use something else besides clinical tests if we are going to use pilot studies to find out where we are going. Later we can go into some long-range five- or ten-year studies, but over a period of a year or so Dr. Kesel had to work with this method to find these things out. You have to work with something that will show results in six months or a year; you can't wait ten years for it.

DR. BIBBY.—You don't have to wait ten years. I think the point is, in all research, the simplest method is the best method. As for discussion of the accuracy of the method, why discuss—do this, that, or the other thing? I think it raises some doubt, and there is doubt, although I don't question correlation. There is very excellent correlation and very useful correlation. But why bother about the interpretation of lactobacillus counts when if we waited a year or so we could give a more definite picture? In other words, I do believe in the usefulness of the lactobacillus count as a very useful and diagnostic aid, but I think it is a mistake to hang too much onto it in determining what is happening, particularly in critical research. I think a good conservative attitude on the lactobacillus counts is waiting six months or a year. I think that we shall actually go farther in a shorter time.

DR. KESEL.—Our whole premise is based on the fact that the lactobacillus is closely correlated with caries. When we produce an environment in which the lactobacillus does not develop, we have gone a long way in reducing caries activity. It may not be that the lactobacillus is the causative factor, but it is certainly closely associated with it.

DR. BIBBY.—Let's not get on causation, let's skip that entirely. As far as I am concerned it is a straight question of logic. I admit correlation; it is useful and valuable, and clinically it is a great aid. But I think we are guilty of false reason when we try to do a thing right by an indirect route when it can be done by direct route. In all research the simplest procedure is the best; the indirect is less reliable than the direct. Let's take an indication; let's not bother about technics. And so far as an indicator is concerned we have a good one. Let's use it and regard it as an indication, 80 per cent or better perhaps, and wait six months or another year until we get clinical evidence. I think, as I said, we are going to make progress more rapidly than if we did not get both sides. I think Dr. Kesel, incidentally, perhaps gave a rather good example of over-reliance on the lactobacillus count. The rest of the flora wasn't examined. There may have been changes more significant than in the case of the lactobacilli that he discussed. So, I think that we make negative progress when we place too much reliance on that sort of research. I don't want to get into causation of dental caries because I do believe it is a valuable clinical aid, and the point I want to make is one of straight logic for what I believe is a more productive type of research. Don't put too much dependence on an indirect measure when a direct measure is available.

DR. KESEL.—Another virtue of the lactobacillus count that those of us in clinical research have found is that it gives the patient a tangible measure of what is going on in his mouth. He becomes much more interested in the control program than he would on a program where he would have to wait for a year or two to determine whether cavities did or did not develop.

DR. BIBBY.—What I had in mind is that it is a valuable clinical aid.

DR. EASLICK.—Dr. Jay, do you agree that the simplest technic is always the best in research? Would you care to discuss that statement?

DR. JAY.—No, I wouldn't say that, but I wish that Dr. Bibby wouldn't be so worried about this. There are many who have objected to laboratory aids in clinical diagnosis, but as medicine progresses there will be used all along, more and more.

DR. BIBBY.—It doesn't worry me necessarily. I should like to make the point. I wouldn't have made it if the discussion hadn't appeared as to whether the method were accurate or not. I am concerned, Dr. Jay, about the lactobacillus count.

DR. ROBINSON.—I am perfectly satisfied now because Dr. Bibby has apparently changed his mind in the last three minutes. He asked why use it at all, in the first place. Now he says that he will use it; so I am satisfied.

DR. EASLICK.—Dr. Armstrong, do you wish to talk?

DR. WALLACE D. ARMSTRONG.—I should like to ask Dr. Bibby which of these methods is more accurate, lactobacillus counts or clinical examination. I have a feeling

that neither one is particularly accurate from the standpoint that I would take as a chemist, because I am told that dentists who examine the same mouth may come out with slightly different results. I wonder if perhaps they aren't both wrong?

DR. BIBBY.—Dr. Armstrong has a very good grasp in any field of logic. I have to admit that it is probably impossible to measure one uncertainty by using an uncertain measuring rod. I wish this thing hadn't come up in lactobacillus discussion. I am worried about lactobacillus because while we do have some fairly good clinical studies of a more or less statistical nature in which one examiner checks against another examiner, we do not have yet, to my knowledge—and I have tried to get some—lactobacillus acidophilus counts over a sufficiently long period of time established on what I consider to be (I am not a statistician), I will say, on what statisticians consider to be reliable statistical basis. Mr. Chairman, I am now off the subject.

DR. EASLICK.—We shall drop the subject, then, Dr. Bibby, and introduce the next speaker.

It is a pleasure to have presented next in this Conference, the information that is available on the utilization of the antibiotics, the drugs which are receiving so much publicity as therapeutic agents at the moment. The man who is elected to discuss this information couldn't send me a very long list of references because there doesn't seem to be a long list of references available. It is a pleasure to present the man who, I think, has done most of the research, at least, who has done considerable research, with these drugs as caries-control agents. Dr. Thomas J. Hill, I find, always presents his subject well and entertainingly. He comes from Western Reserve University in Cleveland where he serves as Professor of Pathology. The subject assigned to him is "The Utilization of Penicillin and Other Antibiotics in the Control of Caries." Dr. Hill, you have thirty minutes in which to present it.

THE USE OF PENICILLIN IN DENTAL CARIES CONTROL

THOMAS J. HILL, SCHOOL OF DENTISTRY, WESTERN RESERVE UNIVERSITY, CLEVELAND, OHIO

May we define first the term "antibiosis," which means the association of two organisms, one of which is detrimental to the other. Antibiotics, then, are the products of organisms which when in contact with other organisms may inhibit the normal biological process of this second organism. This is not a new subject by any means. The probabilities are that this was well recognized by Pasteur, and some antagonism of one organism to another in the oral cavity certainly was recognized by Miller who did at least one classic experiment on the effect of some oral organisms on the growth of induced organisms. It is quite probable that it was also recognized by Black who made the statement "that the logical inference is, that the differences in the susceptibility to caries of different individuals is in something operating in the oral fluids and acting upon the active cause of caries, intensifying or hindering its effect." There has been no really concentrated investigations on these antibiotics until the last few years, but they promise to open up a very promising field for future dental research. My interest in this was induced by the Food and Drug Administration who brought up the problem of what attitude should be taken if a penicillin dentifrice were put on the market. As a result, a rather preliminary experiment was undertaken with a small number of students to see the effect that it might have on lactobacillus counts.

Ten well-selected students, students who would follow our instructions very carefully and very conscientiously, were given a dentifrice which was composed of:

calcium carbonate	74 Gm.
sodium bicarbonate	2 Gm.
tricalcium phosphate	15 Gm.
neutral soap	6.5 Gm.
saccharin, essential oils to make 100 Gm.	

To this was added 1,000 units of penicillin per gram of tooth powder. There was no basis for that 1,000 units; it was just a hit in the dark. But a most striking effect resulted from the use of the dentifrice. These ten students had at the beginning of the experiment an average lactobacillus count of approximately 72,000 per student. At the end of one week the count dropped to 18,000 and kept on dropping for five straight weeks, until it hit about 300. They were kept on penicillin for one more week, and then were taken off penicillin and allowed to use other commercial dentifrices. The count remained at an average of 300 for three months, and then during the fourth month the count immediately jumped back to approximately where it had been originally. They were put back on the penicillin dentifrice and again their counts dropped, somewhat the same as they did the first time, although much more slowly.

At the same time this was going on, McClure, at the National Institute of Health, was conducting an experiment on the use of penicillin in rats. He administered to these rats 75 units of penicillin in each gram of food, and 75 units in each cubic centimeter of water, which gave them a total intake—I am estimating—of about 3,000 units a day. If it is fair to compare that on the basis of body weight, it would be equal in a human being to something in the neighborhood of a little over a million units a day. McClure found that the rats had complete inhibition of dental caries, although he used a fairly large quantity of penicillin in proportion to the body weight of a rat.

One of the problems which was of importance was the keeping quality of our material. We made up various dilutions, some of which were in dehydrated powder and others which were not in dehydrated powder, some of which we refrigerated and others which we did not refrigerate. The effectiveness of this powder was determined by the area of inhibition produced around wells containing the powder in Petri plates that had been seeded with lactobacilli. The keeping qualities of this powder were investigated periodically for a year. The area of inhibition of 150-day-old tooth powder is equal to the freshly made batch. This process of testing was continued, and it took about one year before there was the slightest loss of inhibition in the smallest quantity used, that is, 0.125 unit.

In the measurement of the sensitivity of various strains of lactobacilli to penicillin, there was a good deal of variation. The study was done by a well method and by the measurement of the clear areas of inhibition produced. All lactobacilli were found to be sensitive to penicillin but there was much variation in different strains. By this method we determined also the smallest amount of the drug necessary to inhibit lactobacillus growth. When 2.5 units of penicillin were placed in each well, the area of inhibition varied for different strains of lactobacilli from a diameter 2.3 to 3.3 centimeters. One strain of lactobacillus showed areas of inhibition around wells when one unit of penicillin was placed in each well, but no inhibition when 0.5 unit was used. Other strains produced areas of inhibition of 1 cm. in diameter when 0.125 unit of penicillin was placed in each well. In general, the strains which had the greatest areas of inhibition when 2.5 units were placed in the wells showed the greatest sensitiveness to 0.125 unit of penicillin.

An experiment was conducted with rats fed a Hoppert diet, some receiving plain water, others the same diet with varying amounts of penicillin in the water, and some having sucrose and penicillin added to the water. The rats were fed for 110 days. Seventy per cent of the control rats had dental caries and 50 per cent of the rats receiving 5 units of penicillin per cubic centimeter of water supply had dental caries. Weighted value was given to the carious lesions on the basis of the amount of tooth structure which was destroyed with values of 1 for incipient caries, 2 for deep caries of the dentin, and 3 for caries which involved pulp exposures. By this method the caries-control rats had a weighted value of 0.154, and the rats receiving 5 units of penicillin, a weighted value of 0.135. The rats receiving sucrose showed quite a different result. While 70 per

cent of the control rats had carious teeth, 100 per cent of those receiving additions of 1.5 per cent of sucrose to the water had dental caries. Of the rats that received additions of 5 units of penicillin per cubic centimeter and 1.5 per cent of sucrose to the water, 68 per cent had carious teeth. When similar weighted values were given to the carious lesions, the rats receiving sucrose additions had a caries weight of 0.353, and those with penicillin and sucrose, a caries weight of 0.166. The percentage of carious teeth was also markedly reduced from 22.5 per cent in the rats receiving sucrose to 9.3 per cent in the rats receiving sucrose and penicillin.

Zander has shown, in experiments on hamsters, the results of the use of dentifrices containing penicillin on the incidence of dental caries. One group of animals received no local medication; in the second group, the teeth were brushed daily with a nonmedicated commercial tooth paste; and in the third group a tooth paste containing 1,000 units of penicillin per gram was used. The control animals receiving no medication had 8.76 carious teeth per animal. Those whose teeth were brushed with a commercial dentifrice had 3.87 carious teeth per animal, and those whose teeth were brushed with the penicillin dentifrice had 0.86 carious teeth per animal.

Zander tested also the effect of penicillin, added to saliva, on the rate of acid formation. Controls, consisting of 1 c.c. of a 20 per cent solution of glucose and 4 c.c. of saliva, when incubated produced a pH drop from a pH of 6.5 to a pH of 4 in four hours of incubation. When varying amounts of penicillin (from 10 units to 1,000 units) were added to the saliva before incubation, no reduction in the pH values was observed. In addition, he tested the value of penicillin as a mouthrinse to study the length of time that the value of penicillin was maintained in the saliva. After use of a mouthrinse of 1,000 units of penicillin in 20 c.c. of water, he observed no production of acid on salivas that were collected within two hours and incubated with glucose following the use of the mouthwash.

We initiated an experiment in an orphanage to study lactobacillus counts on a larger scale and the effect that would be obtained on the control of dental caries. An orphanage of boys who were housed in cottages was selected. There were forty boys in each cottage. The odd-numbered cottages were put on penicillin and the even-numbered used as the control group. The boys ranged in age from 7 to 14 and were not a very cooperative group. Perhaps this experiment represents not what could be done with the use of penicillin in carefully selected patients but what might happen if such medication were given to the general public. Two and three lactobacillus counts were made on each boy before the experiment was started and each was classified as negative, plus 1, 2, 3, and 4 degrees of susceptibility. The boys brushed their teeth once a day only (if they brushed them) and they have not followed instructions too carefully. On the other hand, most of the boys are following instructions about as well as can be expected of boys of that age. The following results were obtained after a five-month period: Twenty per cent of the control group and 26 per cent of the experimental group were constantly negative; i.e., they were negative when

we started and remained negative throughout the whole experiment. Thirty-one per cent of the control group and 22 per cent of the experimental group retained the same degree of susceptibility. Sixteen per cent of the control group and 4 per cent of the experimental group had increased susceptibility; thirty-three per cent of the control group and 48 per cent of the experimental group had decreased susceptibility. Nine per cent of the control group had decreased negative counts and 21 per cent of the experimental group had decreased negative counts.

If those who had been negative throughout the whole experiment are eliminated and consideration be given only those who were susceptible to begin with, the results are as follows: In the control group, 21 per cent had increased susceptibility in some degree and 43 per cent had decreased susceptibility. In the experimental group, 65 per cent had decreased susceptibility and only 4 per cent had lactobacillus counts that went up to a different degree of susceptibility. The experiment is still under way and will be continued for some time to determine whether or not there has been a reduction of caries.

After lactobacillus counts, fourteen students were selected who had fairly marked susceptibility to dental caries. Those who had negative counts and those who had low or questionable counts were eliminated, and the experiment was started with a highly susceptible group. It was thought that perhaps penicillin would be more effective in the control of lactobacilli if the penicillin were used in high concentration and then dropped to a lower amount. These boys are given lozenges of 500 units, one every night before retiring, and they are using penicillin dentifrice as well. This experiment is still in progress. At the beginning, five had ++++, one had +++, five had ++, and one had + susceptibility. At the end of one week the lactobacillus counts indicated one with a ++, four with a +, and nine with negative lactobacillus counts. After the lozenges were discontinued but the tooth powder continued for two more weeks, one showed ++++ count, one had +++ count, two had ++, six had + count, and four were negative. We are trying to make a rather careful analysis of what is happening to some of the other mouth flora and what changes are going on in the mouths where the gram-positive organisms are being destroyed. One of the most significant changes that we find is an increase in the presence of the *Aerobacter aerogenes*. At the beginning of the experiment none of the fourteen salivas grew *A. aerogenes* on tomato-juice agar or on Levine's agar. At the end of the third week six salivas grew *A. aerogenes*.

To go back to our rat experiment, before we sacrificed these rats we made smears from their mouths. This is a very inaccurate way of determining mouth bacteria, but rats are not particularly cooperative and it is about the only way it can be done. We used sterile cotton to swab their mouths and inoculated plates for the presence of acidophilus and for the presence of *A. aerogenes*. The correlation is not good, but it is rather indicative. As the rats' mouths showed decreases in the acidophilus counts, increases in the frequency with which *A. aerogenes* were present were also found. This experiment was carried a little further, particularly in relation to the bacterial antagonisms that might be

present between these two particular organisms. Petri plates seeded with acidophilus were used and into the well in the center was fitted a little celloidin cup through which diffusion and dialysis would occur. These plates were seeded with *A. aerogenes* and incubated with broth culture of lactobacilli in the center of the celloidin cup. A bacterial antagonism was found which produced a clear area of inhibition around the wells in the agar seeded with *A. aerogenes*. But, if the procedure was reversed and the *A. aerogenes* put in the center cup and the lactobacilli on the outside, no inhibition occurred unless the pH of the media was sufficiently high to be unfavorable to the growth of the lactobacilli. This does not negate Kesel's experiment with the filtrate. He has shown that the *A. aerogenes* filtrate has an inhibitory effect against the acidophilous. We were working with the organisms themselves and not the filtrate. We have found also that a number of strains of streptococci will inhibit acidophilus, the well method being used.

SUMMARY

1. The oral lactobacilli are sensitive to penicillin and the sensitivity to penicillin varies in degrees depending upon the strain.

2. There is good evidence that the lactobacillus count of the mouth can be reduced by the use of penicillin in one form or another.

3. There is evidence that the incidence of dental caries can be reduced in rats and hamsters by the use of penicillin, but as yet evidence is not available that a similar reduction will occur in human beings. In other words, the use of antibiotics of this kind is very definitely in the experimental stages.

From our experience, we can conclude that if it becomes desirable to use penicillin, its value, like many of the other materials that have been suggested, will depend entirely upon complete and conscientious cooperation of the patient. Such methods will not become good public health methods for the control of dental caries. In other words, the control of dental carries will ultimately depend upon the finding of the immunological factors and the antibiotic factors that are natural to an immune mouth and upon their induction to produce a state of resistance in the patient.

DR. EASLICK.—Dr. Hill's paper is open for discussion.

DR. F. A. ARNOLD, JR.—I am thinking, Dr. Hill, of one of the first slides that you put on, which showed a rapid drop in acidophilous counts in a small group of students following the use of penicillin. It showed an abrupt rise after four months. Now, did the counts for the whole group go up in similar character after four months, or do you have any explanation of why there is a four-month period there?

DR. HILL.—No, none whatever. The whole group went up. Some of them went up very much more than others. There were one or two in that group that did not rise very greatly, but there was none whose count did not go up to some degree.

DR. ALLEN O. GRUEBBEL.—Dr. Hill, what is the likelihood that the lactobacilli might become penicillin-fast as in the case of some other types of bacteria?

DR. HILL.—I think there are two questions that I imagine will be asked: the question of penicillin-fast organism, and the question of allergic manifestations. I shall answer Dr. Gruebbel's question as best I can to begin with. I think that perhaps we have not a very accurate understanding of what is meant by penicillin-fastness, or by penicillin-resistant strain of organisms. As a matter of fact, the whole thing is more or less hypothetical. But I should like to quote and read just one paragraph, if I may, from Kolmer's *Penicillin Therapy*, where he discusses this, and I shall just read these two or three sentences as an explanation of penicillin-fastness.

> The mechanism is unknown, but Demerac believes that this resistance is not induced by the action of penicillin on bacteria, but originates through mutation. In other words, according to this hypothesis, when penicillin is added to a culture in low concentration it eliminates non-resistant or susceptible cells but may leave some individuals possessing a natural resistance to the compound.
>
> According to this hypothesis, therefore, it is assumed that there are a number of genes in organisms that effect resistance to penicillin, and if any of them mutates, the individuals in which such mutation occurs acquire resistance. On the other hand, however, there is evidence to show that resistance is always natural and not acquired. Under these circumstances it is assumed that in any culture of an organism penicillin may eliminate the susceptible organisms without eliminating a resistant variant which continues to be resistant indefinitely without acquiring any additional resistance as the result of exposure to the compound.

In other words, if they are resistant strains to start with, they will be resistant strains when one gets through, and if they are not, they won't be.

I am sure the question of allergic manifestations will be asked, and so I might as well answer it while I am on my feet. We have used penicillin in perhaps a little over 400 people, and they have been using penicillin for varying degrees of time, some of them as long as one and one-half years. Up to the present time we have not had a case of allergy. I think we shall get them; as a matter of fact, I suspect that there will be a certain percentage of people who will become sensitized to the use of this material. We must also remember that the sensitization to penicillin is not nearly as undesirable as is the sensitization to the sulfa drugs. The probablities are that if these patients become sensitized to it, the worse that will probably happen to them will be a dermatitis and itching skin in varying degrees, most of which will not be too severe.

DR. EASLICK.—Thanks, Dr. Hill. I should like to testify, however, that the itching skin is terrible. From two experiences with penicillin I don't want any more of it.

DR. ROBERT M. STEPHAN.—I don't know whether Dr. Hill answered Dr. Arnold's question, but as I recall, on the slide that Dr. Hill showed there was a rapid drop in the lactobacillus count following the use of penicillin; then the penicillin was discontinued and the count stayed down for a few more months. . . .

DR. HILL.—Three months.

DR. STEPHAN.—. . . and then all of a sudden some of the counts jumped up for some reason; all jumped together at a given time. In other words, could you discontinue penicillin and then wait for two or three months and say they all had gone up together, or did some jump up earlier than others and some later, and so forth?

DR. HILL.—No, these counts were made weekly for the first eight weeks and then monthly for four months. Between the third and fourth month the counts went up in varying degrees, some of them not as much as others.

DR. STEPHAN.—So, you could get three months of negative counts after discontinuation of penicillin in all cases?

DR. HILL.—We did in these cases.

DR. STEPHAN.—Yes.

DR. NED B. WILLIAMS.—Dr. Hill, did you measure saliva to determine how long there remained any active penicillin in saliva after the dissolution of the 500 unit lozenges?

DR. HILL.—Yes.

DR. WILLIAMS.—Did you find how long that activity was present in saliva after the lozenges?

DR. HILL.—Yes.

DR. WILLIAMS.—And also did you do that with the dentifrice? Did you discover how long after the specified time of brushing the saliva would remain potent for your test organism?

DR. HILL.—Yes, we measured them with lozenges, and we have measured them with dentifrice. We found that the length of time the penicillin is demonstrable in the saliva varies with different individuals and it will run from about thirty minutes to an hour, perhaps around forty-five minutes being about the average.

DR. WILLIAMS.—With the 500 units?

DR. HILL.—With 500 units of penicillin lozenges or with a dentifrice containing 500 units.

DR. WILLIAMS.—May I ask what the test organism was?

DR. HILL.—Type II lactobacillus.

DR. HAMILTON B. G. ROBINSON.—What I had to say wasn't about penicillin, but about a very important point, I think, that was brought out in Dr. Hill's paper. That is, the fact that 43 per cent of the controls in one of these studies showed a decrease in their caries activity as indicated by lactobacillus counts. Now, let us suppose, instead of using penicillin (which apparently was effective) Dr. Hill had been using "Lydia E. Pinkham" compound without any control and had reported results. He would have reported 43 per cent decrease in the lactobacillus count or caries activity. That is what I would like to call, using someone else's term, "institutional effect." We must have definite controls. I think the evaluating committees should analyze carefully these papers that have to do with the control of dental caries. Are there adequate controls? For example—this is a commercial—the best way of getting clinical controls that I know is reported in a paper on the Coast Guard study by Arnold and his co-workers* made on so-called matched human "litter-mates." If you want to find out how you can get good controls, study that paper and then consider the other papers that you have read to see if they are really adequate controls. I think that is a beautiful point that Arnold and his group brought out in their paper.

DR. HELMUT A. ZANDER.—Dr. Hill mentioned that he was a little bit worried about the cooperation at this institution, and I am just wondering whether the 43 per cent may not be possibly accounted for by the fact that some of the boys started trading dentifrices? I don't know—maybe I shouldn't be suspicious, but since he mentioned that somebody didn't use it at all, and that they weren't too cooperative, and knowing boys' institutions, I wouldn't be surprised if someone wanted to see how the material tasted.

DR. HILL.—I hardly think, Dr. Zander, that was the case. They were housed in different cottages; none of them knew who was on the experiment and the institution didn't know. I don't believe that is the case. I think it is just the natural lack of cooperation in youngsters.

*Arnold, F. A. Dean, H. T., and Singleton, D. E., Jr.: Effect of Caries Incidence of Single Topical Application of Fluoride Solution to Teeths of Young Adult Males of Military Population, *J. D. Res.* 23: 155, 1944.

DR. EASLICK.—There will be an eight-minute recess. Please come back promptly.

(Recess)

Now, if all you hard-working students and faculty will come to order, please, it is time to begin the complex evaluation of the use of fluorine as a caries-control measure. We have scheduled this morning the first of the three speakers on this phase of caries control, and the first speaker is Dr. Francis A. Arnold, Jr., who comes from the National Institute of Health. The job that has been assigned to him by the Planning committee is an "Evaluation of the effectiveness as Caries Control Measures of Ingested Fluorides in Water, Food, Bone Flour, and Proprietary Preparations." Dr. Arnold, I personally shall be surprised if you do not do a very excellent job in the next forty-five minutes.

AN EVALUATION OF THE EFFECTIVENESS AS CARIES CONTROL MEASURES OF INGESTED FLUORIDES IN WATER, FOOD, BONE FLOUR, AND PROPRIETARY PREPARATIONS

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I do not think that it is possible this morning for us to talk on the subject of evaluation of fluorides in dental caries control as far as water supplies, food, and other preparations are concerned because we do not have sufficient data with which to evaluate the use of such materials. At present, we have considerable evidence from epidemiological studies showing wide variations in the amount of dental caries in fluoride and nonfluoride areas. Whether or not such data are directly transferable to support the use of fluorides for caries control by the methods mentioned is only an assumption.

First, I should like to go back a little into the history of the hypothesis that fluorides have an effect on dental caries. In the latter part of the nineteenth century references to the use of fluorides for the control of dental caries are found. At that time some workers in Europe were feeding calcium fluoride tablets to mothers during pregnancy for the protection of their teeth and the teeth of the offspring. We have no data with which to evaluate the results of any of this work. Since this is a conference on the disease of dental caries and its control, we wonder *why* they fed the fluorides. I think their reason for feeding fluorides at that time was the idea of building a resistant tooth structure on the basis that a fluorapatite is more resistant to decomposition than other forms of apatite.

Later, in this country, McKay[27, 28] started studying a disease of the teeth which was called "mottled enamel." In these studies, McKay and also Black repeatedly commented on the fact that the teeth in endemic areas of mottled enamel, in spite of their poorly calcified structure, were no more susceptible to dental caries than were teeth in nonendemic areas. In addition, Bunting, Crowley, Hard, and Keller[8] from Michigan, when reporting on a study of mottled enamel at Minonk, Illinois, stated that caries did not appear to progress the same in mottled teeth as in normal teeth. It should be noted that at the time they were studying areas where the fluoride content of the water was in an excessive range and the degree of mottling produced was of the moderate-to-severe type.

After the determination of the cause of mottled enamel, in about 1931, Dean[9] started working to determine the safe limits of fluorine in a water supply. Through these studies the quantitative relationship between concentration and effect has been established. Today, we know how much mottled enamel or dental fluorosis to expect in a population group depending upon the amount of fluorine present in the domestic water supply. We know the safe limits of fluorine permissible in a water supply in order to eliminate dental fluorosis as a public health hazard, i.e., about 1.5 ppm of fluorine. In establishing these safe limits, Dean was of the opinion that dental caries was lessened in areas where one did not

observe the moderate and severe types of mottled enamel. Thus it was decided to conduct specific epidemiological studies on this dental caries-fluorine relationship to determine the lower limits of fluorine which would still be effective.

In all the studies to which I shall refer, the observations were made on a select group of individuals. First, the communities in which the studies were conducted were selected on the basis of having used their water supplies for a period greater than the lives of the individuals who were examined. Only those children 12 to 14 years of age who were born and reared in these communities were examined.

Severe mottled enamel* is representative of the type of tooth structure present in areas where the water contains excessive amounts of fluorides. As McKay,[27, 28] Bunting[8] and others pointed out, these teeth, in spite of their defective structure, are no more susceptible to dental caries than are normally calcified teeth.

Very mild mottled enamel shows only slight evidence of any disturbance in enamel formation. Usually the tips of the premolars and the canines have a characteristic white opaque appearance which occasionally is also observed on the incisors. This represents the greatest amount of "mottling" that will occur in an area where the water supply contains 1 ppm of fluorine.

During the first fluorine-dental caries study in Illinois[12] a considerable difference in the amount of dental caries observed in the 12- to 14-year-old children at Galesburg, where the water supply contains 1.8 ppm of fluorine, was noted when compared with children in Quincy where the water supply is fluorine free. The Quincy children show a caries experience rate which is more than three times the rate observed at Galesburg. This difference occurred in spite of the fact that more teeth were filled in the Quincy children than the total caries experience rate of the children at Galesburg.

Another point of special interest in this study came from a comparison of the prevalence of caries in Galesburg children who showed macroscopic dental fluorosis with those children showing no evidence of the disease. About 50 per cent of the children examined in Galesburg showed macroscopic exidence of fluorosis. The caries prevalence rate on this group of children was found to be little different from the caries rate of those children showing no positive evidence of fluorosis. This observation suggested the possibility that lower levels of fluoride would also be associated with decreased caries rates. This hypothesis was verified in the studies to be discussed later.

In addition to the dental examinations in this study,[12] lactobacilli counts were made to determine whether or not there was a difference in the oral flora in these areas. A considerable difference in the percentage distribution of the lactobacilli counts was observed in Galesburg and Quincy. In Galesburg, about 28 per cent of the children had negative counts and another 10 per cent had counts under 100 lactobacilli per cubic centimeter of saliva. In Quincy, there were only 12 per cent with negative counts and about 8 per cent with less than 100 per cubic centimeter of saliva. These low lactobacilli counts are characteristic of mouths in which dental caries is inactive. On the other side of the

*For pictures of mottled enamel the reader should consult J. A. D. A. 30: 1278-1283, 1943.

scale in the "high count" range, the percentage distribution is the opposite. Almost three and one-half times as many Quincy children have counts of 30,000 and over per cubic centimeter of saliva as do the Galesburg children. These high counts are characteristic of the oral flora in mouths where caries is very active. These results suggest that the "activating force" of dental caries varies in these two communities.

A study in eight suburban Chicago communities[13] gave additional evidence. Elmhurst, where the domestic water supply contains 1.8 ppm. fluorine, had a caries rate of about 250. Maywood and Aurora, whose water supplies have 1.2 ppm fluorine, had caries rates of similar character. Joliet, with 1.3 ppm fluorine in the water supply, showed a caries rate of 300. Elgin, with 0.5 ppm fluorine, had a caries rate of about 450. Evanston, Oak Park, and Waukegan, all using fluoride-free water supplies, showed caries rates ranging from 670 to 820. There is a considerable difference in the amount of dental caries in the children of these different Chicago suburbs. It would be difficult to assume from an epidemiological standpoint that these differences in caries rates were caused by variations in the dietary, other than water, of the people in Maywood as compared with those of Evanston and Oak Park. They are neighboring suburbs and the people are buying their foods out of the same type of store. On the basis of economic levels, as a group, the people in Evanston and Oak Park might be expected to have better nutrition than individuals in Maywood. Evanston and Oak Park represent two of the wealthiest suburbs in this country.

We should consider not only that there is less caries in the fluoride areas than in the nonfluoride areas, but also how the caries picture varies. First, I should like to point out the difference in the amount of caries on the proximal surfaces of the maxillary incisors. In the fluoride areas we found about 0.6 per cent to 1.3 per cent of the proximal surfaces carious, in the intermediate fluoride area (Elgin) about 4 per cent, and in Evanston, Oak Park, and Waukegan we found 10, 9, and 18 per cent, respectively. In other words, there is a ten- to fifteenfold difference in the amount of caries on the proximal surfaces of the anterior teeth in the fluoride areas as compared with the nonfluoride areas. I do not think that we should interpret these results as an indication that fluorine has a selective action on anterior surface caries. We must also consider that this result may be another measure of the difference in the activating forces of dental caries in the different areas.

Two other observations of special interest in the Chicago study are the differences in the loss of first permanent molars and the amount of dental fluorosis. In the fluoride communities we found from twelve to about twenty first molars lost per 100 children examined, but in the fluoride-free cities these rates ranged from thirty-one in Oak Park to about eighty in Waukegan. Three to four times as many first molars are lost in the fluoride-free areas by the time the children have reached 14 years of age.

The amount and degree of dental fluorosis are of major importance in this problem. The incidence of dental fluorosis varies from about 40 per cent in Elmhurst to 0.2 per cent in the fluoride-free area of Waukegan. As may be noted, only 15 per cent of the Aurora children showed even the slightest evidence of fluorosis. Aurora is characteristic of areas where the water supply contains

the optimum amount of fluorine. I might also add that this mild degree of fluorosis is of no esthetic significance and is found, as a rule, only on the tips of the canines and second molars.

In all the epidemiological studies conducted at the National Institute of Health, with the cooperation of State Health Departments and the University of Michigan,* 8,576 12- to 14-year-old white children from twenty-seven cities in eight states have been observed. The over-all picture is like that shown for the suburbs of Chicago; where the water supplies contain fluorine we find low dental caries rates, and where the water supplies are fluorine free we find high caries rates. I should also like to call special attention to the results in some of the cities that have been studied. For example, in Herford (Deafsmith County, Texas) we found a very low caries rate; about 150 DMF teeth per 100 children. It has the lowest rate of all twenty-seven cities, but it has about 100 per cent incidence of fluorosis. In Colorado Springs we find a dental caries rate of around 240, but we still find over 70 per cent of the people showing dental fluorosis. The hopeful part of this problem is that in the areas where the water supply has about 1 ppm of fluorine and where only very mild dental fluorosis occurs in 10 to 15 per cent of the individuals, there is almost as much inhibition of dental caries as observed in the high fluoride areas. In other words, there is little evidence that it would be of any great value to increase the fluorine content of a water supply above about 1 ppm for dental caries control.

I should also like to call attention to the results in some of the cities such as Vicksburg, Mississippi, Nashville, Tennessee, and Clarksville, Tennessee. These communities show some evidence of dental fluorosis and a decreased caries rate, yet we can find little evidence of fluorine intake as far as their water supply is concerned. Such results should be considered when the use of fluorides in water supplies for caries control is being evaluated. It may be that 1 ppm of fluorine is not required in areas of the Southeastern and Southwestern part of our country to bring about the desired effect.

Next I shall discuss the results of 2,329 lactobacilli counts† made on selected groups of children in some of the twenty-seven cities considered in the epidemiological studies. First, I want to point out the differences observed in the low counts. In the fluoride areas we find 38 per cent of the children showing counts of zero or less than 100 as compared to 18 per cent in the fluoride-free communities. Conversely, in the high-count range (20,000 or more lactobacilli per cubic centimeter of saliva) we find the percentages reversed. Less than 30 per cent of the children in the fluoride areas have high counts as compared to over 50 per cent of the children in the fluoride-free areas.

What has been presented so far represents a summary of the evidence accumulated to date in regard to how water supplies may affect dental caries rates. At present, studies are in progress to determine the effect on dental caries of adding sodium fluoride to a level of about 1 ppm fluorine to common water supplies. Theoretically, we should get the same results as have been observed in natural fluoride areas at a similar fluoride level. We have no direct evidence that such a change will occur. We do have experimental evidence on rats and

*J. A. D. A. 36: 32, 1948 (Table 2).
†See Table I, p. 44, ref. 2.

some limited evidence in human beings which suggest that "treated" fluoride waters act the same as natural fluoride waters. It may be that we shall have to wait until we rear a new generation of children to at least 10 or 12 years of age on a fluoride-treated water before we have the final answer.

Another method of caries control suggested by the results of these epidemiological studies would be to duplicate "field" conditions on an individual basis. This would mean increasing fluoride ingestion by daily supplements of fluorides either in the food or through individual water supplies. This can be done by the use of natural "high fluoride content" products such as bone meal or preparations of fluorides in the form of tablets. As most of you know, such products are on the market today. According to the calculations of McClure,[22] it would take about one-half of a milligram of fluorine a day in children up to about 3 years of age, and children from 3 to about 8 or 9 years of age should receive about one milligram of fluorine per day. These calculations are based on the average amount of water that would be drunk in fluoride areas at these age levels, and the amount of fluorine that would be contained in water of an optimum fluoride content.

We have limited evidence to indicate that such a procedure of feeding fluoride supplements would only be necessary for about the first eight years of life. The results of studies at Bauxite, Arkansas,[12] showed caries to be inhibited in those individuals whose teeth were formed on fluoride waters but erupted into a fluoride-free water environment. The results reported by Deatherage[16, 17] on selectees from Illinois also support this assumption.

McClure and Kinser[23] studied the relation of fluorine in the drinking water to the fluorine content of urine. There is a direct and very close correlation between the fluorine content of the urine and the fluorine content of the water supply. There is no evidence in these results of any excessive fluoride storage in the body. In fluoride-free areas the fluorine content of urine was at a rather constant level, 0.3 to 0.4 part per million. In other words, the amount of fluorine contributed by foods in various sections of the country remains at a rather constant low level.

A study on fluoride excretion was made by McClure, Mitchell, Hamilton, and Kinser[25] on five individuals living under strictly controlled environmental conditions. Their total fluorine intake was measured and compared to fluorine excretion in urine, feces, and body sweat. When fluorine was added to a fluoride-free water supply, such as Urbana water, it was excreted in the same manner as when the fluorine source was a natural fluoride water, such as Galesburg water. When cryolite or bone meal are used as the source of fluorine, there is a different excretory picture. Apparently a higher percentage of the fluorine from these sources is not readily absorbed and passes through the body and is eliminated in the feces. Such results must be considered when one is attempting to control caries by daily fluoride supplements.

The final part of this problem which I should like to discuss, briefly, deals with the toxicity of fluorine. This discussion will concern only nondental toxicity and only those levels of fluorides commonly present in water supplies. As many of you know, there are reports in the literature of detrimental effects from what is apparently water-borne fluorine. These reports are from areas in South

Africa and in India. In evaluating the findings in these areas we should consider the conditions under which the people of these countries live. These results may not be applicable to this country.

In many regions of the United States people are using waters which contain more than 1 ppm of fluorine*; some of the waters having 8 to 15 parts per million. Naturally, all the areas in this country using fluoride waters are not known, but as state health departments make routine fluorine analyses more areas will be known. However, I think we should remember that in spite of the large number of people using these fluoride waters, we have had no reported detrimental effects in this country other than on the teeth. Also, McClure[24] has reported on the prevalence of bone fractures in residents of numerous fluoride and nonfluoride areas. He was unable to demonstrate any significant difference between the areas in this respect. This suggests that there is no skeletal damage of consequence in areas where the fluorine content of the water supply is no more than about 4 to 5 parts per million.

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*See Fig. 2, p. 9, ref. 1.

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DISCUSSION

DR. EASLICK.—I should like to thank Dr. Arnold very much, formally, and then I should like to ask for discussion.

Does anyone wish to discuss this evaluation period of fluoride?

DR. HAMILTON B. G. ROBINSON.—I want to ask a question. Dr. Arnold, would you care to discuss the suggestion of Weaver who studied adults and children in high- and low-fluoride areas and suggested simply a delaying effect in dental caries rather than true control of caries?

DR. ARNOLD.—We have made a study on a very limited number of individual adults in Bartlett, Texas, and Courtland, Virginia, and we found fewer DMF teeth in the adults in these fluoride areas than has been reported for similar ages in other areas in the United States. Deatherage's figures on selective service inductees showed a definite inhibition of dental caries in those selectees who came from fluoride areas. These selectees ranged from 21 to 28 years of age. That is about the extent of reported evidence that we have in this country. Naturally, using a DMF rate for comparing differences you are going to find the differences decreasing with age. After a tooth becomes carious and is listed as a DMF tooth it does not change from that. Thus, as we go into adult groups the comparative differences decrease.

DR. EDWARD TAYLOR.—Dr. Arnold, I have two or three questions I should like to ask. I am thinking now about the optimum concentration of fluorine we can use safely so far as mottling of enamel is concerned and for the maximum of inhibition of caries. First, I should like to ask you what the percentage of mottled enamel was at Galesburg. You may answer these as I ask them.

DR. ARNOLD.—About 50 per cent of the children at Galesburg showed some evidence of dental fluorosis, and 50 per cent were listed as normal.

DR. TAYLOR.—Now, what I want to ask you is if you know what percentage of them had brown stains rather than just the white opaque enamel.

DR. ARNOLD.—I think that we might have observed a slight brown pigmentation in one or two of the Galesburg children. Typical "brown stain" in the Galesburg children is certainly not very prevalent. You do not start observing brown stain until you get in areas with 2 or 2.5 ppm of fluorine or more. It is necessary to have a little more disturbance in calcification than occurs at Galesburg before the secondary phenomenon, called brown stain, is observed.

DR. TAYLOR.—I agree with you; that is the point I wanted to bring out. I believe that many people when speaking of mottled enamel, are thinking in terms of brown stain. You made the statement that you found 100 per cent of the children in Deaf Smith County had mottled enamel. I should like to know how many children had continuous residence and whether you were speaking of white mottled enamel rather than brown stain?

DR. ARNOLD.—I think I understand what you are trying to bring out here, and it is an important point. If I am not mistaken, we examined about sixty individuals who had continuous residence in Herford. Brown stain is prevalent in Herford, but, I don't know exactly what the percentage incidence is. It wouldn't run over about 20 or 25 per cent, the white chalky type of fluorosis being more common.

DR. TAYLOR.—One more question then, please, sir. Personally, I don't think the percentage of brown stain would even be that high, but that is my own personal opinion. I wanted to ask you if it isn't a fact that with an additional 0.5 ppm of fluorine, I mean by that a total of 1.5 ppm, you get an additional inhibition of caries in the neighborhood of 20 to 30 per cent.

DR. ARNOLD.—Well, the only thing that I can say is that there is little difference in caries rates, as I showed you on the slides, between Colorado Springs at 2.5 ppm and Aurora at 1.2 parts per million. I think the difference is something like a caries rate of about 250 to a caries rate of about 280. As far as we have observed, about 90 per cent of the benefit is obtained at around 1 ppm of fluorine, and this concentration is well below the range of having a chance of getting any bad effects from the standpoint of fluorosis. Dental fluorosis begins to be a health problem when the water contains between 1.5 and 2 ppm of fluorine. In other words, I don't think it would make enough difference in the caries rate to justify decreasing our margin of safety.

DR. SIDNEY B. FINN.—Dr. Arnold, I believe you said you found the greatest reduction in caries in the four upper anterior teeth. Now, could this not possibly be due to the fact that fluorine in the drinking water comes in contact with the upper anterior teeth more than it does with the other teeth, and there might be a topical effect?

DR. ARNOLD.—No, I didn't want my statement to be interpreted to mean that there is a "selective action" of fluorine for the anterior teeth. It may be due to what I shall call a function of numbers. You can't have a caries rate of 800 until the anterior teeth are involved because you wouldn't have enough carious teeth. So I don't think we can say at present that it is a selective action. We do have this evidence: at Bauxite, Arkansas, we had children whose teeth were formed on fluoride water, but had erupted into a nonfluoride environment. Still, the proximal surfaces of the anterior teeth also showed this wide difference in caries when compared with the same teeth at Benton. They didn't have a topical application of fluorine from the water supply because they were using fluoride-free water after the teeth erupted.

DR. BENJAMIN M. MEDOF.—I should like to ask Dr. Arnold if the minerals and chemicals, with the exception of fluorine, in the water at Aurora were similar to those at Minneapolis, would the doctor then expect to find the same results?

DR. ARNOLD.—I think that question is partly answered in studies by McClure and his associates in which they produced what you suggest. Fluorine was added to Urbana water, which is a natural surface water containing those elements normal to surface water, and compared to Galesburg water, which is a natural fluoride water. They didn't show any difference so far as excretion of the fluoride is concerned. If you will recall the slide showing the results in twenty-seven cities, these communities were using all types of water. As far as other elements in the water being important in this problem, I don't doubt that they are, but as for the usual mineral constituents we should remember that those twenty-seven cities included communities that had hard water with or without fluorine and cities that had soft water with or without fluorine. Colorado Springs is using a water supply which is melted snow and has less than 40 ppm of total solids in it. About twenty years ago this would have passed for distilled water. It does have fluorine in it and the effect on caries is the same as in regions where we have highly mineralized waters. Experimentally, you can stop caries in rats by feeding sodium fluoride in distilled water. That is about the total of all the evidence at the present time. How or what other elements of water supplies may affect this problem, we do not know.

DR. HELMUT A. ZANDER.—My remarks are related to the question Dr. Finn raised a minute ago, that there was a greater apparent difference between the caries attack on the proximal surfaces of the anterior teeth than on the molars; that is, ten to fifteen times more proximal caries where there was no fluorine in the water supply, as compared to those areas having fluorine in the water supply. Now, I am just wondering whether this could be related to the severity of carious susceptibility? Is this condition similar to those cases of limited caries susceptibility where the first permanent molar is attacked while the other teeth are not, and conversely, when we have a great amount of caries susceptibility the first permanent molar and the other teeth are attacked. In other words, could one imply that fluorides alter, in part, the caries susceptibility, so that the caries picture is similar to one where the susceptibility to dental caries is decreased? I wonder if I made myself clear?

DR. ARNOLD.—I don't think this part of the problem is at all clear, Dr. Zander. I don't think with the type of examination used and from the manner in which the data have been processed, that we can actually answer what you asked. I think to answer this problem it would be necessary to compare similar surfaces of teeth with the same posteruptive exposure in a comparable oral environment.

DR. ZANDER.—I intend to discuss the very same problem tomorrow in relation to the use of silver nitrate. We have a very similar problem there, and it may be worth while to see a comparison between the problem in the fluoride instance as compared with that in silver nitrate applications.

DR. ROBINSON.—On this same subject, I think that part of the answer that you want is available in a study made by Arnim.* He took the data of R. Leigh Wood and of Hyatt and determined the percentage of caries occurrence for each tooth in the mouth at various ages. That, of course, showed the most susceptible areas to be the occlusal surfaces of the molars, the upper anterior teeth being much less susceptible and the lower anterior teeth the least susceptible. So, if we should apply some method that would very slightly reduce caries, the first place we should show the reduction would be in the lower anterior teeth; a better method would reduce caries in the upper anteriors. If we used a perfect method we would even stop caries in the occlusal surfaces of the first molars. I think that is the point that you are driving at, isn't it?

DR. ZANDER.—Yes.

DR. EASLICK.—Is there any further discussion?

DR. PHILIP E. BLACKERBY.—Dr. Arnold, my question is in regard to the use of fluorine for people who have individual water supplies, and there are many such people in this

*Arnim, S. S.: Utilization of Biologic Principles in the Practice of Operative Dentistry. II. Statistics, an Aid to Efficient Diagnosis, *J. D. Res.* 21: 243, 1942.

country. Is there any evidence to indicate that the daily ingestion of a single glass of water containing the 1 mg. of fluorine, which I believe was recommended as the requirement, will have any different effect from that of the ingestion of a natural water containing fluoride, or will it involve any danger to the consumer? In other words, water containing 1 ppm of fluorine consumed throughout the day might conceivably have a different effect from that of a single glass of water containing 1 mg. of fluoride?

DR. ARNOLD.—I can only answer a part of that question, Dr. Blackerby. One milligram of fluorine in a glass of water would not be a toxic dose of sodium fluoride by any manner or means, and there is no evidence of any hazard from a chronic toxicity standpoint. Whether it will have a similar effect, or any effect, on dental caries is not known at present.

DR. BLACKERBY.—There is no reason to believe there can be any serious danger involved in the general use of sodium fluoride tablets?

DR. ARNOLD.—No, I see no reason to believe there would be, as long as they are used within the prescribed safe limits.

DR. GENEVIEVE STEARNS.—Dr. Arnold, did I misunderstand you? Did you say that there was no evidence of skeletal deposition of fluorine in these communities that are using fluoride waters? That would be quite different from the known facts in animals. Beef-bone always runs considerably higher in fluorine than veal-bone in any analyses I have seen. The fluorine must have been definitely absorbed. It couldn't have been excreted in the urine or sweat, or it would be quite at variance with all we know about metabolism. Did I misunderstand you, or did you make that statement?

DR. ARNOLD.—Well, you misunderstood the intent of what I said. Surely, there is fluorine in the skeletal system and I don't doubt whatsoever that the fluoride content of bones in fluoride areas would be increased. The evidence from Dr. McClure's work which I presented here showed that we could account for about 90 per cent of the fluoride ingested in water in these areas by the amount of fluorine excreted in the urine and the body sweat. There was no evidence of any storage of fluorine to an extent that we might expect pathological changes in the bone. That is what we would be worrying about. I should like Dr. Armstrong to comment on this point if he would.

DR. WALLACE D. ARMSTRONG.—I was troubled by the same thought that Dr. Stearns brought up, but I was going to discuss it in private with Dr. Arnold, since I didn't think it would be a point of general interest to the group. We have been able to show that by feeding drinking water containing 20 ppm of fluorine to rats the fluorine content of the skeletal system increases ten- or twentyfold. I think we should repeat this experiment and analyze the whole skeleton so as to see what fraction of the administered dose was retained in this manner. As a matter of fact, in my experience, the determination of fluorine in urine and in feces is extremely difficult, if not impossible. The results are usually on the high side, more fluoride being found in the urine to which a definite amount of sodium fluoride has been added than is actually present. So, I have never quite been able to understand the results that McClure obtained which indicate the excretion of nearly 100 per cent of the administered dose. We should also consider the fact that the subjects were receiving fluoride in materials other than in the water, so that the apparent positive 100 per cent excretion may not amount to that.

It would be interesting, I think, to obtain some bone from an autopsy specimen in the fluoride areas, and I dare say that you would find the content of those bones to be much higher than in nonfluoride areas. As a matter of fact, Dagmar and Wilson in England have done just that.